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VOLUMEN VIII

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Fasc. 3. (pag. 169—252) edit.: 25—IX—1957
Fasc. 4. (pag. 253—328) edit.: 20—XII—1957

INDEX AUTORUM

Ajello, L., 3.
Andleigh, H. S., 135, 138.
Baum, G. L., 189, 272.
Benedek, T., 1, 85, 86, 87, 168, 252, 327.
Benedek, Th. G., 84.
Bingham, E. L., 189.
Chavez Bastista, A., 89, 103, 127.
Ciferri, R., 169, 183.
Clark, F. M., 243.
Fain, A., 54.
Fernandez Vital, A., 89.
Floch, H., 194.
Friedman, L., 47.
Georg, L. K., 3, 249.
Giordano, A., 169.
Gonçalves de Lima, O., 89.
Herin, V., 54.
Hoeven, L. H. van der, 253.
Kaden, R. H., 260.
Kitamura, K., 164.
Kleine-Natrop, H. E., 154.
Meyer, J., 239.
Muftic, M. K. S., 18, 121.
Negroni, P., 216.
Parini,F., 27.
Redaelli, P., 169, 183.
Ribotto, L., 27.
Rubel, H., 189.
Scherr, G. H., 62, 73.
Schwarz, J., 189, 272.
Silva Maia, H. da, 103.
Smith, C. E., 47.
Sprovieri, L., 27.
Stillians, A. W., 161.
Vermeil, C., 206.
Vries, G. A. de, 83, 154, 253.
Wang, C. J. K., 189.

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CONTENTS: CONTENUTO: INHALT: TABLE DES MATIÈRES:

Pag.

Tibor Benedek: Editorial 1

Libero Ajello & Lucille K. Georg: In vitro Hair Cultures for Differentiating between Atypical Isolates of *Trichophyton mentagrophytes* and *Trichophyton rubrum*. (*Communicable Disease Center, Public Health Service, U.S. Dept. of Health, Education and Welfare, Chamblee, Ga.*) 3

Mahmoud K. S. Muftic: *Blastomyces cerolytica* (sp.n.) and its Relation to Coccidioides. (*Mycol. Lab., Middle Euphrat Hospital, Kuffa, Iraq*) 18

F. Parini, L. Ribotto & L. Sprovieri: Pneumomicosi cronica (Aspergillosi?) con generalizzazione pluriviscerale. (*Pio Ist. S. Corona, Sanatorio G. Salvini; Divisione Rivalta; Ist. Maffucci*) 27

Lorraine Friedman & C. E. Smith: The Comparison of Four Strains of *Coccidioides immitis* with Divers Histories. (*Naval Biol. Lab., Dept. of Bact. and School of Public Health, Univ. of California, Berkeley, Calif.*) 47

A. Fain & V. Herin: Deux cas de Rhinosporidiose nasale chez une oie et un canard sauvages à Astrida (Ruanda-Urundi). (*Lab. médical, Astrida, Ruanda-Urundi, Congo Belge*) 54

George H. Scherr: The Effect of Hormones on Experimental Moniliasis in Mice. I. Sex Hormones, Cortisone and Somatotrophic Hormone. (*Dept. of Microbiol., The Creighton Univ. School of Med., Omaha, Nebra.*) 62

George H. Scherr: The Effect of Hormones on Experimental Moniliasis in Mice. II. Gonadotropins. (*Dept. of Microbiol., The Creighton Univ. School of Med., Omaha, Nebra.*) 73

Libri novi 83

A. Chaves Batista, Oswaldo Gonçalves de Lima e **Albino Fernandes Vital:** *Aspergilus Gracilis Bainier, var. Sartoryi* (Biourge) Batista, Lima e Vital n. var. Sua importância filogenética. (*Instituto de Micologia, Universidade do Recife, Pernambuco, Brasil*). 89

A. Chaves Batista e **Heraldo da Silva Maia:** Estudo taxonómico de um novo Aspergillaceae *Aspergillus Fasciculatum Batista e Maia* n.sp. (*Instituto de Micologia, Universidade do Recife, Pernambuco, Brasil*) 103

Mahmoud K. S. Muftic: Mutation of Mycobacteria to Proactinomyces by radioactive cobalt. (*Mycological Laboratory, Middle Euphrat Hospital, Kuffa, Iraq*) 121

A. Chaves Batista: Alguns Agaricaceae saprófitos de Pernambuco. (*Instituto de Micologia, Universidade do Recife, Pernambuco, Brasil*) 127

H. S. Andleigh: In vitro study of Antifungal activity of Pentamidine and Stilbamidine. (*Dept. of Pathology and Bacteriology, Sawai Man Singh Med. Coll., Jaipur, India*). 135

H. S. Andleigh: Etiology of Maduromycosis in India. (*Dept. of Path. and Bacteriol., S.M.S. Med. Coll., Jaipur, India*) 183

G. A. de Vries und **H. E. Kleine-Natrop:** Sporotrichum cerebriforme nov. spec. (*Centraalbureau voor Schimmelcultures, Baarn, The Netherlands*) 154

A. W. Stillians: In memoriam Frederick DeForest Weidman 161

Kanehiko Kitamura: In memoriam Masao Ota 164

Tibor Benedek: Editorial 168

R. Ciferri, P. Redaelli (†) **& A. Giordano:** Revisione di alcuni *Cephalosporium* isolati da casi patologici umani. (*Ist. ed Orto Bot., Pavia*) 169

R. Ciferri & P. Redaelli (†): *Trichosporium mantegazzae* Poll. e *Cladosporium chlamydeum* n.sp. (*Ist. ed Orto Bot., Pavia*) 183

Jan Schwarz, Gerald L. Baum, C. J. K. Wang, Eula L. Bingham & Harold Rubel: Successful Infection of Pigeons and Chickens with *Histoplasma capsulatum*. (*Jewish Hosp. and the Lab. of Mycology, Cincinnati Gen. Hosp., Cincinnati, Ohio*). 189

Pag.

Hervé Floch: Revue critique des investigations et de la littérature mycologique pour les années 1946—1956 en Guyane Française. (*Inst. Pasteur, Cayenne, Guyane Franç.*) 194

Claude Vermeil: Mycologie médicale humaine en Tunisie: revues des principales acquisitions, analyses des données concernant les années 1946—1956. (*Inst. Pasteur, Tunis*) 206

Pablo Negroni: Desarollo de los estudios micológicos en la Argentina en el último decenio. (*Centro de Micología, Facultad de Ciencias Médicas, Buenos Aires*) 216

Jacques Meyer: In Memoriam August Sartory 239

F. M. Clark: In Memoriam Fred Wilbur Tanner 243

Lucille K. Georg: In Memoriam Rhoda W. Benham 249

Libri novi 252

C. A. de Vries & **Ludolph H. van der Hoeven:** Report of a case of black grain maduromycosis of the foot caused by an aberrant strain of *Madurella mycetomi* (Laveran) Brumpt on Curaçao, Netherlands Antilles. (*Bureau voor Schimmelcultures, Baarn and Columbus, Ohio, U.S.A.*) 253

Rudolf H. Kaden: Precipitin Studies for the Identity of Antigens in Different Species of Sporotrichum. (*Hautklinik, Freie Universität Berlin*) 260

Jan Schwarz & **Gerald L. Baum:** Critical Review of Medical Mycology in the United States 1946—1956. (*Clin. Labs., Jew. Hosp. and Lab. of Mycol., Cincinnati Gen. Hosp., Cincinnati, Ohio, U.S.A.*) 272

Libri novi 327

GALLERY OF CONTEMPORARY NOTED MYCOLOGISTS

1. JOHANNA WESTERDIJK, Baarn.
2. SELMAN A. WAKSMAN, New Brunswick, N.J.
3. ALDO CASTELLANI, Lisbon.
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11. N. F. CONANT, Durham, N.C.
12. JUAN E. MACKINNON, Montevideo.

PLATES: TAVOLE: PLANCHES: TAFELN:

pag.

I. PIERO REDAELLI IV/1

II. RAFFAELE CIFERRI IV/1

III. TIBOR BENEDEK IV/1

IV. M. K. MUFTIC, Mutation of Mycobacteria 120/121

V. FREDERICK DEFOREST WEIDMAN 160/161

VI. MASAO OTA 164/165

Gallery of Contemporary Noted Mycologists. Nrs. 1, 2, 3, 4 . . 168/169

VII. AUGUST SARTORY 238/239

VIII. FRED WILBUR TANNER 242/243

IX. RHODA W. BENHAM 248/249

Gallery Nrs. 5, 6, 7, 8 . . . . 252/253

Gallery Nrs. 9, 10, 11, 12 328

101 text illustrations

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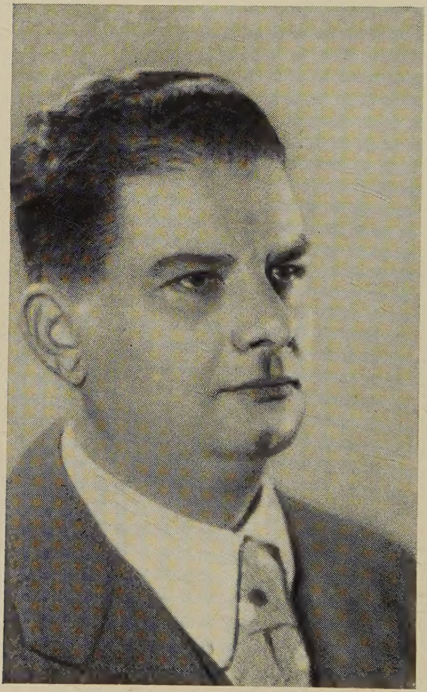
(1898—1955)

Founder-Editor
1937—1955



RAFFAELE CIFERRI

Founder-Editor
1937—1955

TIBOR BENEDEK

Editor
1956—

EDITORIAL

Following the untimely death of one of the founder-editors, Piero Redaelli, and the resignation of the other, Raffaele Ciferri, I had the great honor to be appointed as Editor of *Mycopathologia et Mycologia Applicata.*

This appointment, however, carries with it great obligation and high responsibility. Ciferri and Redaelli created a journal which became an open forum for uncensored investigations, free discussions of new ideas and theories with prejudice toward none. We shall carry on in their spirit to honor their unstinted effort, their signal achievement and to continue making this journal the center of international mycological publications.

We shall not be afraid of controversial subjects, theories or ideas. From whatever quarter they may come, only the substance shall count and not the author's standing. Free research and true progress can thrive only in the open. New findings, new ideas may thrive under the sun or wilt on their own vines. Time alone shall be the great judge. There shall be only one firm criterion by which to judge contributions: the methodological knowledge of the author and the command of the proper tools of research.

In the period of 1937 to 1955 a great number of excellent contributions has been published in this journal in almost every field of pure and applied mycology as well as in related fields from almost every country of the world where serious mycological research is carried on. In the hope that we will be able to keep up the great standard of *Mycopathologia et Mycologia Applicata* set by Ciferri and Redaelli, we will carry on with determined courage and unfailing endeavor.

Tibor Benedek

IN VITRO HAIR CULTURES FOR DIFFERENTIATING BETWEEN ATYPICAL ISOLATES OF *TRICHOPHYTON MENTAGROPHYTES* AND *TRICHOPHYTON RUBRUM*

LIBERO AJELLO, Ph. D. and LUCILLE K. GEORG, Ph. D. ¹)

Chamblee, Georgia

The morphological inconstancy of the dermatophytes has been the source of much taxonomic confusion. Narrow concepts of species limits have led to the creation of over 200 "species", the great majority of which have been defined on tenuous criteria.

However, growing realization that a given dermatophyte may vary considerably in its morphological and biological properties has stimulated taxonomic revision of this group of keratinophilic fungi. Today most medical mycologists recognize only 14 species as valid: *Trichophyton concentricum, T. ferrugineum, T. gallinae, T. megnini, T. mentagrophytes, T. rubrum, A. schoenleini, T. tonsurans, T. verrucosum, T. violaceum, Microsporum audouini, M. canis, M. gypseum, Epidermophyton floccosum.*

Of these, *T. mentagrophytes* and *T. rubrum* are two common dermatophytes with basically distinct cultural characteristics. The first generally produces clavate macroconidia, globose or subglobose microconidia and spirals of tightly wound mycelium. The second gives rise to long macroconidia with parallel side walls and microconidia that are elongate in form. Most isolates of this latter species, in addition, produce a deep red pigment when grown on Sabouraud dextrose agar, while a great many isolates of *T. mentagrophytes* elaborate brownish pigments on the reverse of the colony.

However, the range of cultural variations within these two species is so great that considerable overlapping occurs in their gross and microscopic morphology as well as in pigmentation. It is not unusual to encounter isolates of *T. rubrum* that fail to produce red pigments or conversely some cultures of *T. mentagrophytes* that do elaborate a deep red pigment. Clavate macroconidia are borne by some isolates of *T. rubrum* (Fig. 1A, B), and certain isolates of *T. mentagrophytes* have been found to form pencil-shaped spores (Fig. 2–A, B).

The difficulties inherent in identifying atypical forms of *T. mentagrophytes* and *T. rubrum* have been recognized by several investigators. In a detailed study of the cultural expression of

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¹) From the Communicable Disease Center, Public Health Service, United States Department of Health, Education, and Welfare, Chamblee, Georgia.

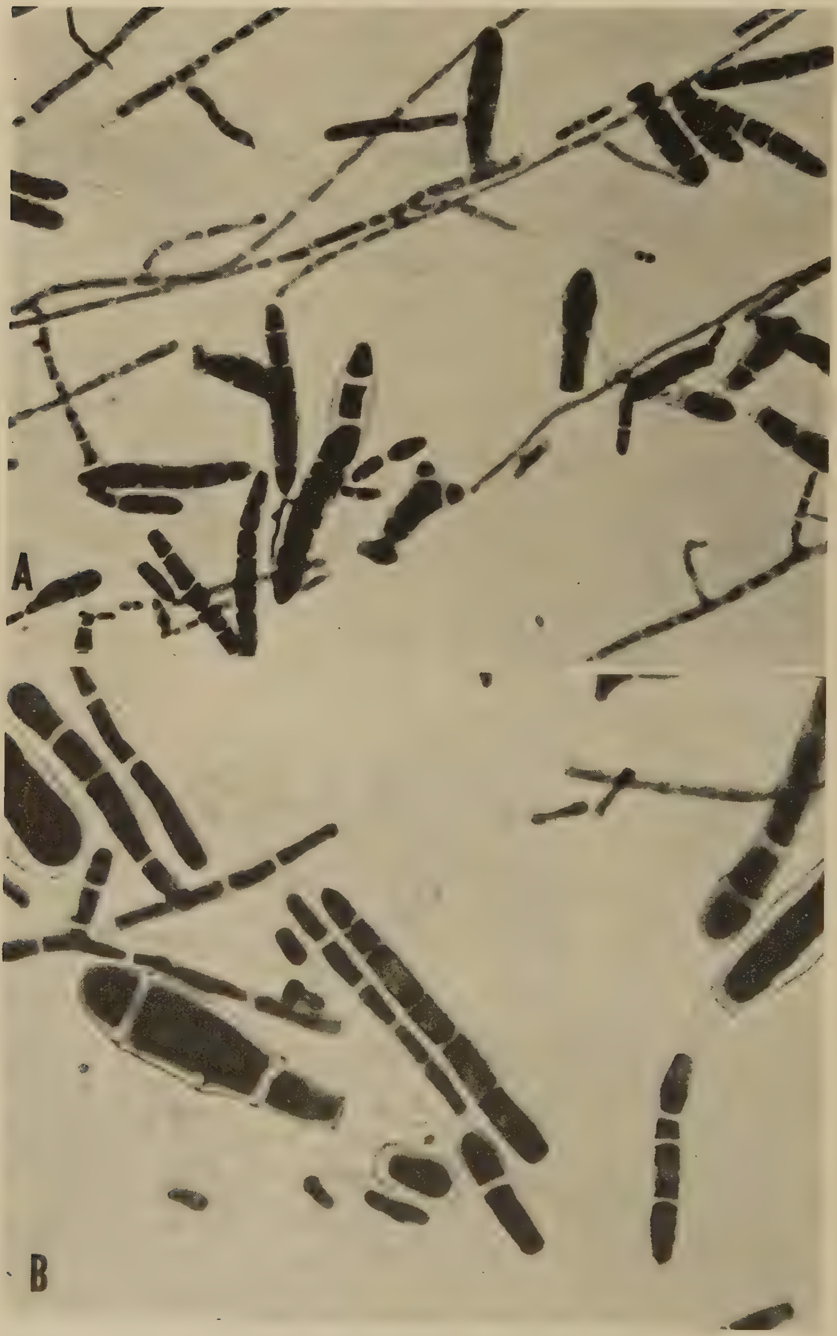

Figure 1. A, B. Group of predominately clavate macroconidia produced by *Trichophyton rubrum* A8. Original magnification (A) × 475; (B) × 970.

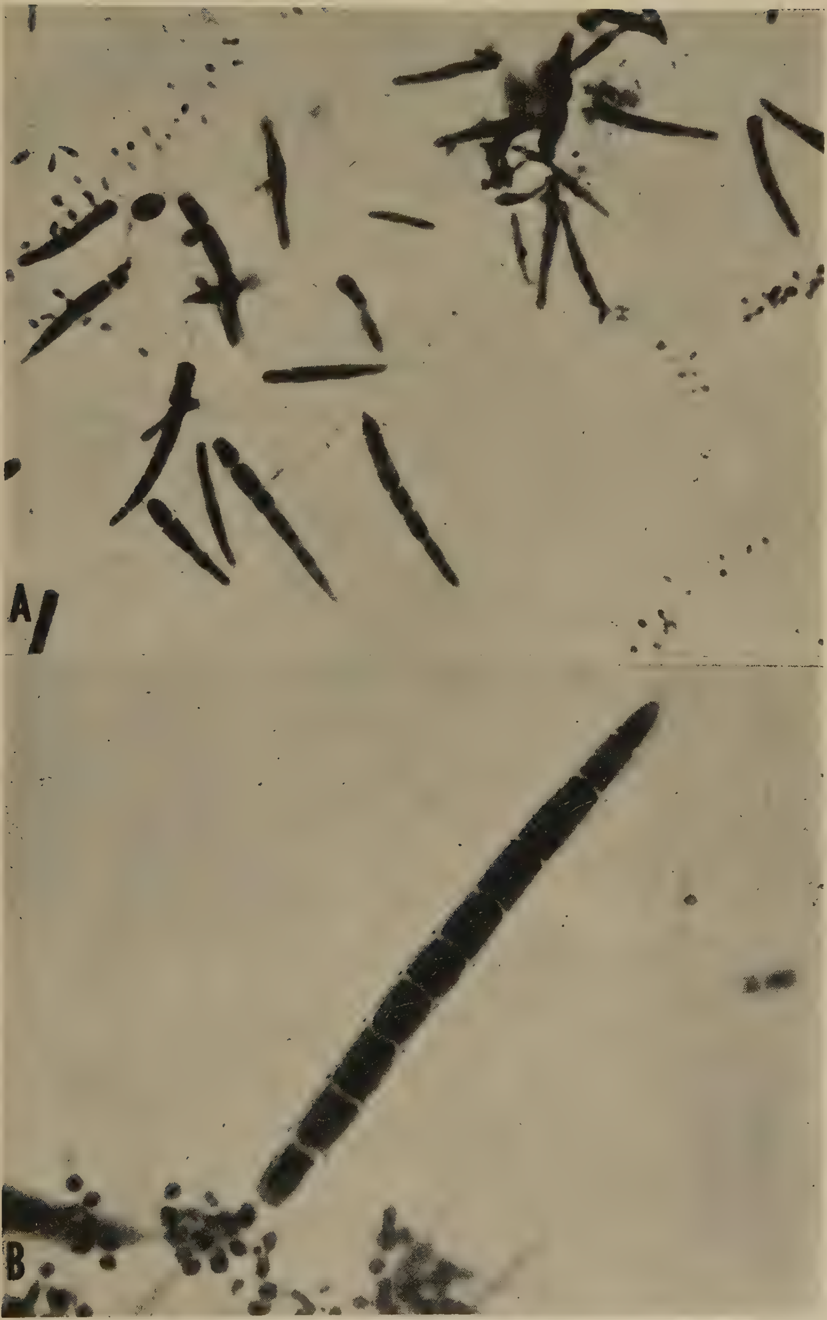

Figure 2. A. Pencil-shaped marcroconidia formed by *T. mentagrophytes* A4. Original magnification × 475.

B. A single pencil-shaped macroconidium. Original magnification × 970.

several isolates of *T. mentagrophytes* and *T. rubrum* on 7 different culture media, EDGECOMBE (1942) concluded that the growth characteristics of these two species on potato dextrose agar were definitive. However, observations made on a large series of isolates of these two dermatophytes grown on this medium have failed to reveal any consistency in pigment formation or spore development.

Heart infusion agar enriched by tryptose was considered by BENHAM (1948) to be of great value in the identification of *T. rubrum*, for on this medium 49 of 50 isolates of *T. rubrum* produced "characteristic macroconidia". But again difficulties have arisen since isolates of *T. rubrum* are encountered from time to time that fail to form macroconidia on that medium.

BOCOBO and BENHAM (1949) recognized the "inconsistent behaviour" of *T. mentagrophytes* and *T. rubrum* and recommended cornmeal agar enriched with 1 % dextrose as an aid in separating the two species. On this medium, *T. rubrum* would develop a pink or red pigment while *T. mentagrophytes* would not develop pigment. Unfortunately, many isolates have failed to conform to this pattern and isolates of *T. mentagrophytes* have been obtained that form pink, red, or yellow pigments on cornmeal dextrose agar; while several isolates of *T. rubrum* have produced yellowish rather than red pigments on that medium. These aberrant forms could be identified as to species only by the combination of structures and/or pigments produced on a wide variety of media.

It occurred to one of us (L.A.) that the manner by which detached filaments of hair were attacked by keratinophilic fungi would be of value in identifying dermatophytes. In 1934 DAVIDSON and GREGORY (1934) noted that hair filaments exposed to *T. mentagrophytes* were radially penetrated by organized groups of hyphae forming wedge-shaped perforations. More recently, VANBREUSEGHEM (1949) demonstrated that the different dermatophyte species varied in the manner in which they attacked hair filaments *in vitro*. The members of one group behaved in the manner described by DAVIDSON and GREGORY for *T. mentagrophytes*, i. e., they formed "perforating organs", while other species brought about a gradual erosion of the hair filaments. *T. rubrum* was one of the fungi that did not perforate hair.

The fundamentally distinct manner by which *T. mentagrophytes* and *T. rubrum* attacked hair thus was considered to be of potential value in distinguishing atypical forms of these two fungi from one another. The manner of testing the value of this diagnostic criterion and the results obtained are the subject of this presentation.

Materials and Methods

The cultures used in this study came from many sources. Some were recent isolates while others had been maintained for varying periods of time in our culture collection. Thirteen isolates of *T. mentagrophytes*, twelve of *T. rubrum*, and fifteen cultures of forms not readily identifiable as either of these two species were studied.

Morphological observations were made on all the fungi used. Sabouraud dextrose agar [1]) was selected to determine their gross colony features and pigmentation as this medium is generally used as the standard for such descriptions by medical mycologists. Other media, however, including wort agar, potato dextrose agar, cornmeal agar, oat meal agar, and heart infusion tryptose agar (Blood Agar Base, Difco), were used to induce spore production. Cornmeal dextrose agar was used according to the method described by BOCOBO and BENHAM (1949) for pigment production.

The study fungi were identified as far as possible on the basis of the features developed on these media. Isolates that differed in minor points, but whose major characteristics conformed to those of one particular species were identified as that species, but they were designated as being atypical. Where the morphological characteristics of a particular isolate were so aberrant that it could not be equated with either species, its identification was deferred.

The manner by which all these fungi attacked hair *in vitro* was studied as follows: Snort segments of human hair were sterilized in Petri dishes by autoclaving them at 120° C. for ten minutes. Following the addition of 25 ml. of sterile distilled water and 2 or 3 drops of 10 % sterilized yeast extract[1], the plates were inoculated with several fragments of colonies of the test fungi that had been grown in tubes of SABOURAUD dextrose agar. Once inoculated, the plates were incubated at 25° C. in a constant temperature box and examined at regular intervals over a period of 21 days. Hair segments were removed from the dishes with sterile forceps, placed in a drop of lactophenol cotton blue mounting fluid and examined under the microscope to determine whether or not they had been perforated. This procedure is considered to be simpler than the one developed by VANBREUSEGHEM (1949) for determing the effect of fungi growing on hair filaments *in vitro*.

RESULTS

Results of the preliminary morphological observations indicated that of the 40 strains studied, it was possible to clearly identify 13 as *T. mentagrophytes*, and 12 as *T. rubrum*. Even among these "typical" strains, there was considerable variation in morphological characteristics and pigment production, however, in each case the preponderance of the characteristics was referable to one of the two species (Tables I and II). The remaining 15 strains were not so clearly identifiable on morphological characteristics and pigmenta-

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[1]) Difco, Difco Laboratories, Inc., Detroit, Michigan. Trade names are used as a means of identifying a product; their use does not constitute endorsement by the U.S. Public Health Service.

TABLE I. *Morphological characteristics of 13 isolates of T. mentagrophytes and their manner of attacking hair in vitro.*

Isolates	GROSS MORPHOLOGY	MICROSCOPIC MORPHOLOGY	Pigment on corn-meal dextrose agar	Morphological Identification	Hair Perforation
	Sabouraud-dextrose agar 1. Topography 2. Surface Texture 3. Surface Pigment 4. Reverse Pigment	*Special Media* 1. Microconidia 2. Macroconidia 3. Spirals 4. Others			
M-1	1. Flat 2. Granular 3. White to tan 4. Rose-brown	1. 4+ globose (wort agar) 2. 2+ clavate (wort agar) 3. 3+ tight (wort agar) 4. —	—	*T. mentagrophytes*	+
M-2	1. Flat 2. Downy 3. Cream to tan 4. Rose-brown	1. 4+ globose (wort agar) 2. 3+ clavate (wort agar) 3. None 4. —	—	*T. mentagrophytes*	+
M-3	1. Flat 2. Powdery 3. White to tan 4. No pigment	1. 4+ globose (wort) agar) 2. 1+ clavate 3+ irregular (wort agar) 3. 1+ tight 4. —	pink	*T. mentagrophytes*	+
M-4	1. Flat 2. Granular 3. White to tan 4. Rose-brown	1. 4+ globose (Sab. dex.) 2. 2+clavate (wort agar) 3. 3+tight (Sab. dex) 4. —	—	*T. mentagrophytes*	+
M-5	1. Flat 2. Downy 3. White 4. No pigment	1. 4+globose (Sab. dex.) 2. 1+clavate (wort agar) 3. None 4. —	—	*T. mentagrophytes*	+
M-6	1. Flat 2. Downy 3. White to tan 4. No pigment	1. 4+globose (Sab. dex.) 2. 2+clavate (wort agar) 3. None 4. —	—	*T. mentagrophytes*	+

M-7	1. Flat 2. Downy 3. White to cream 4. No Pigment	1. 3+globose (wort agar) 2. 2+clavate (wort agar) 3. None 4. —	Yellow	*T. mentagrophytes*	+
M-8	1. Flat 2. Downy 3. White to cream 4. No pigment	1. 2+globose (wort agar) 2. 1+clavate (wort agar) 3. 1+tight (cornmeal agar) 4. —	—	*T. mentagrophytes*	+
M-9	1. Flat 2. Downy 3. White to cream 4. Rose-brown	1. 3+globose (wort agar) 2. 1+clavate (wort agar) 3. None 4. —	—	*T. mentagrophytes*	+
M-10	1. Flat 2. Powdery 3. Yellow to deep pink 4. Rose-brown	1. 4+globose (wort agar) 2. 3+clavate 1+pencil shape (wort agar) 3. None 4. —	Yellow	*T. mentagrophytes*	+
M-11	1. Flat 2. Fluffy 3. White 4. None	1. 2+elongate (wort agar) 2. 3+irregular 1+clavate (wort agar) 3. 1+tight (wort agar) 4. —	—	*T. mentagrophytes*	+
M-12	1. Flat 2. Fluffy 3. White 4. None	Sterile mycelium (previously produced spores typical of *T. mentagrophytes*).	—	*T. mentagrophytes* pleomorphic	+
M-13	1. Flat 2. Granular 3. Cream to tan 4. Rose-brown	1. 4+globose (Sab. Dex. agar) 2. 2+clavate 2+irregular (wort agar) 3. 2+tight (wort agar) 4. —	—	*T. mentagrophytes*	+

TABLE II. *Morphological characteristics of 12 T. rubrum isolates and their manner of attacking hair in vitro.*

Isolates	GROSS MORPHOLOGY	MICROSCOPIC MORPHOLOGY	Pigment on corn-meal dextrose agar	Morphological Identification	Hair Perforations
	Sabouraud-Dextrose Agar 1. Topography 2. Surface Texture 3. Surface Pigment 4. Reverse Pigment	*Special Media* 1. Microconidia 2. Macroconidia 3. Spirals			
R-1	1. Flat 2. Fluffy 3. White 4. Deep red	1. 4+elongate (wort agar) 2. 1+pencil shaped (oatmeal agar) 3. None	pink	*T. rubrum*	—
R-2	1. Irregularly heaped 2. Fluffy 3. White to pink 4. Deep red	1. 4+small globose (wort agar) 2. 3+pencil shaped 1+clavate, irregular (blood agar base) 3. None	pink	*T. rubrum*	—
R-3	1. Flat 2. Fluffy 3. White 4. Deep red	1. 4+elongate (wort agar) 2. 3+pencil shaped (blood agar base) 3. None	pink	*T. rubrum*	—
R-4	1. Flat 2. Fluffy 2. White 4. Deep red	1. 4+elongate (wort agar) 2. 1+irregular (blood agar base) 3. None	pink	*T. rubrum*	—
R-5	1. Flat 2. Fluffy 3. White to pink 4. Deep red	1. 4+elongate (Sab. dex agar) 2. 4+pencil shaped (blood agar base) 3. None	pink	*T. rubrum*	—
R-6	1. Flat 2. Fluffy 3. White to pink 4. Deep red	1. 3+elongate (wort agar) 2. 2+pencil shaped (blood agar base) 3. None	pink	*T. rubrum*	—
R-7	1. Flat 2. Fluffy 3. White 4. Deep red	1. 3+elongate (wort agar) 2. 2+pencil shaped (blood agar base) 3. None	pink	*T. rubrum*	—

R-8	1. Flat 2. Fluffy 3. White 4. Deep red	1. 4+elongate (wort agar) 2. 3+pencil shaped (blood agar base) 3. None	pink	*T. rubrum*	—
R-9	1. Flat 2. Fluffy 3. White 4. Light red	1. 3+elongate (wort agar) 2. None found 3. None	yellow	*T. rubrum*	—
R-10	1. Flat-center folded 2. Fluffy 3. White to pink 4. Deep red	1. 3+elongate (wort agar) 2. 2+pencil shaped (blood agar base) 3. None	pink	*T. rubrum*	—
R-11	1. Flat 2. Fluffy 3. White to tan 4. Red	1. 3+elongate (wort agar) 2. None found 3. None	pink	*T. rubrum*	—
R-12	1. Flat 2. Fluffy 3. Pink to tan 4. Deep red	1. 2+globose (wort agar) 2. 2+pencil shaped 1+irregular (wort agar) 3. None	pink	*T. rubrum*	—

tion. In four instances the majority of the characteristics appeared to be referable to *T. rubrum*; these strains were classified as *T. rubrum* atypical. In three cases, where the resemblance was closer to *T. mentagrophytes*, the strains were called *T. mentagrophytes* atypical. In six cases, it was not possible to decide for one species, and these were listed as questionable (Table III).

All the strains were given code numbers, so that the investigators would not be influenced by the morphological identifications, and *in vitro* hair cultures were made of each isolate.

All of the fungi grew well when inoculated in the Petri dishes containing sterile distilled water and hair. Within a few days, wisps of newly formed mycelium could be observed floating in the water or developing around some of the hair segments. Generally by the tenth day the mycelium became matted around the hair and was readily visible to the naked eye (Fig. 3). Hair perforations were detected in some of the preparations by the fifth day following inoculation; usually, however, these were not found in abundance until the tenth to fourteenth day (Fig. 4 A–C). Gentle heating of the lactophenol cotton blue mounts aided the detection of perforations as heat intensified the staining of the mycelium. The tests

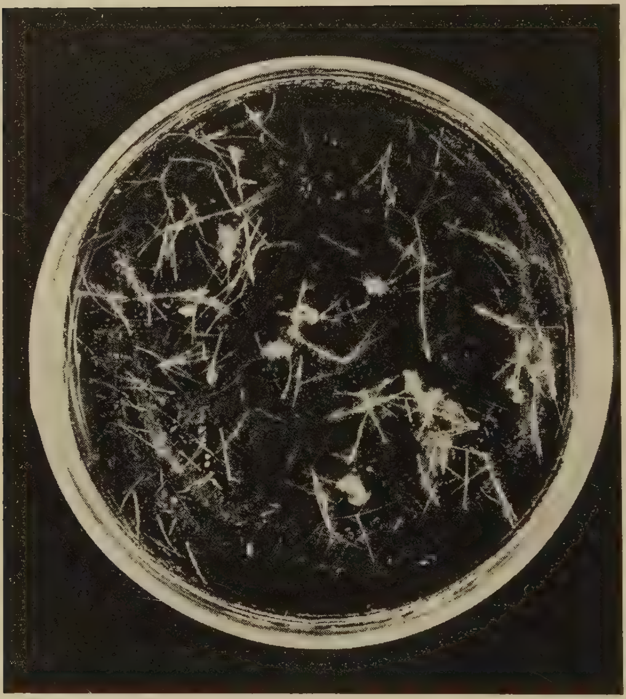

Figure 3. Appearance of *T. mentagrophytes* growing on hair segments in water.

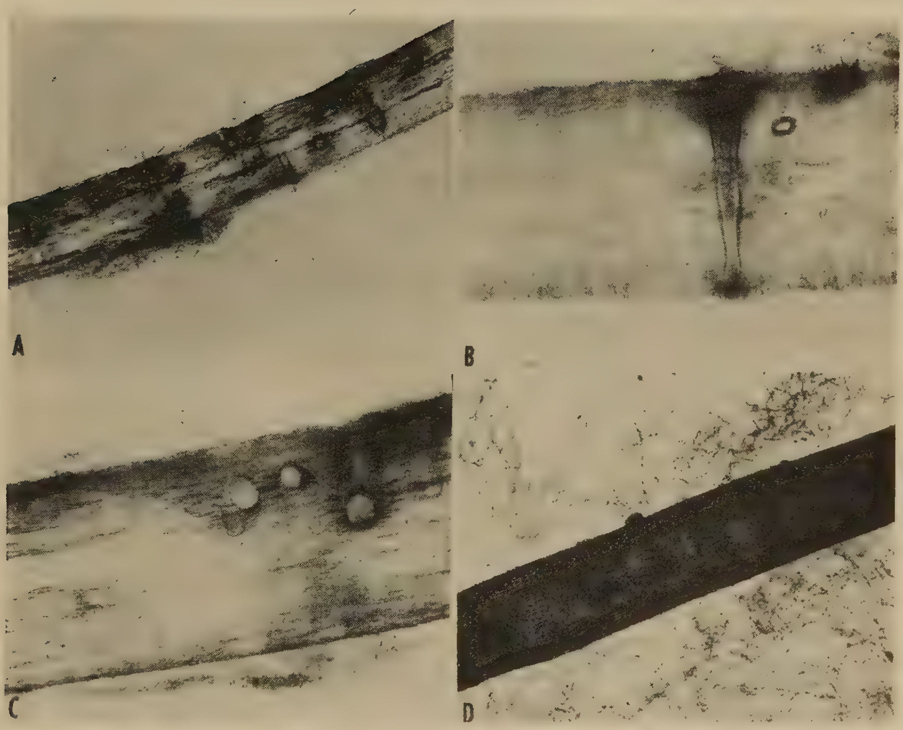

Figure 4. A. Hair segment with multiple perforations caused by *T. mentagrophytes* M10. Original magnification × 220.
B. Median optical view of a perforation extending through the full width of a hair filament. Original magnification × 760.
C. Surface view of a group of perforations. Original magnification × 760.
D. Failure of *T. rubrum* (R5) to perforate hair after 3 weeks of growth. Original magnification × 220.

Table III. *Morphology of 15 atypical isolates not readily equated with either T. mentagrophytes or T. rubrum and their manner of attacking in vitro.*

Isolates	Gross Morphology	Microscopic Morphology	Pigment on cornmeal dextrose agar	Morphological Identification	Hair Perforations
	Sabouraud-Dextrose Agar 1. Topography 2. Surface Texture 3. Surface Pigment 4. Reverse Pigment	*Special Media* 1. Microconidia 2. Macroconidia 3. Spirals 4. Others			
A-1	1. Flat 2. Powdery 3. White 4. Red brown	1. 2+globose 2+elongate (wort agar) 2. 2+pencil shaped (blood agar base) 3. 2+spirals loose (wort agar) 3. —	—	*T. rubrum* atypical	—
A-2	1. Folded 2. Downy 3. White 4. Dark red	1. 2+elongate (wort agar) 2. 3+pencil shaped (wort agar) 3. None 4. —	pink	*T. rubrum* atypical	—
A-3	1. Flat 2. Granular 3. White to tan 4. Dark red	1. 4+globose (wort agar) 2. 1+irregular small (wort agar) 3. None 4. —	pink	?	+ *T. mentagrophytes*
A-4	1. Flat 2. Granular 3. Pink 4. Dark red	1. 4+globose (wort agar) 2. 3+pencil shaped 1+irregular (blood agar base) 3. None 4. —	red	?	+ *T. mentagrophytes*
A-5	1. Flat 2. Fluffy 3. White to tan 4. Red	1. 4+globose (wort agar) 2. 2+clavate 3. 3+tight 4. —	pink	*T. mentagrophytes* atypical	+

A-6	1. Flat 2. Downy 3. White 4. Dark red	1. 3+elongate 2. 1+pencil shaped poorly formed (wort agar) 3. None 4. —	pink	*T. rubrum* atypical	—
A-7	1. Flat 2. Fluffy 3. Deep pink 4. Deep red	1. 3+globose 2. 4+pencil shaped (blood agar base) 3. None 4. —	red	*T. rubrum* atypical	—
A-8	1. Flat 2. Powdery 3. Rose to tan 4. Deep red	1. 4+globose 2. 2+irregular, pencil shaped 2+clavate (wort agar) 3. 2+loose (wort agar) 4. —	pink	?	— *T. rubrum*
A-9	1. Flat 2. Fluffy 3. White 4. Deep red	1. 1+elongate 2. 1+irregular, small (wort agar) 3. None 4. —	pink	?	— *T. rubrum*
A-10	1. Folded 2. Downy 3. Cream to pink 4. Deep red	1. 4+globose and elongate (wort agar) 2. 2+pencil shaped 2+clavate (wort agar) 3. None 4. —	pink	?	+ *T. mentagrophytes*
A-11	1. Flat 2. Fluffy 3. White to yellow 4. Yellow to orange	1. 1+elongate (Sab. Dex. agar) 2. None formed (wort agar) 3. None 4. 4+nodular bodies (cornmeal agar)	yellow	?	+ *T. mentagrophytes*
A-12	1. Folded 2. Downy 3. White 4. None	1. 4+globose (wort agar) 2. 3+irregular 1+clavate (wort agar) 3. None 4. —	—	*T. mentagrophytes* atypical	+ few only formation delayed
A-13	1. Folded 2. Downy 3. White 4. None	1. 4+elongate (wort agar) 2. 1+clavate (wort agar) 3. None 4. —	yellow	*T. mentagrophytes* atypical	+

A-14	1. Flat 2. Fluffy 3. White 4. None	1. 2+globose (wort agar) 2. 3+pencil shaped (blood agar base) 3. None 4. —	yellow	*T. rubrum* atypical	—
A-15	1. Heaped and folded 2. Downy 3. Rose 4. Deep red	1. 2+elongate (wort agar) 2. 3+pencil shaped (wort agar) 1+ varied forms 3. None 4. —	pink	*T. rubrum* atypical	—

were considered negative if no perforations were observed following 21 days of growth.

A study of Tables I and II indicates that the 13 strains previously identified as *T. mentagrophytes* perforated the hairs, whereas the 12 strains which had been identified as *T. rubrum* did not. In Table III it can be seen that those strains which most closely resembled *T. mentagrophytes*, but which were atypical, also perforated the hairs; while the atypical *T. rubrum* strains did not (Fig. 4–D). In the cases of those strains which could not be identified on morphological criteria, the behavior of the fungus in the *in vitro* hair culture was the deciding factor in determing its identity.

Discussion

Examination of the tables reveals that only certain isolates developed morphological characteristics that were exclusively possessed by either *T. mentagrophytes* or *T. rubrum*. It will be observed, however, that when morphological identification was possible the mode of development on hair was consistently of one type. That is, perforating organs were produced by all the isolates which could be morphologically identified as *T. mentagrophytes*, and no perforations were formed by those strains which had been identified as *T. rubrum*. For those few strains which could not be identified on morphological criteria alone, the ability or inability to penetrate hair proved to be decisive in their identification.

It is interesting to note that one of the test cultures of *T. mentagrophytes* (M 12), although completely pleomorphic at the time the *in vitro* hair cultures were made, perforated hair in the manner characteristic of actively sporulating isolates of that species.

It should be emphasized that the ability to perforate hair is not unique with *T. mentagrophytes* and consequently this property cannot be utilized as the sole basis for separating that species from *T. rubrum*. Several other fungi form perforating "organs", among which may be mentioned the saprophyte, *Keratinomyces ajelloi*, the dermatophytes, *M. canis* and *M. gypseum*, and several as yet undescribed fungi (AJELLO) encountered in a wide variety of soils. This phenomenon becomes of value only when correlated with the gross and microscopic features of the fungus undergoing study as manifested on appropriate media.

Summary

Isolates of *T. mentagrophytes* and *T. rubrum* are encountered that cannot be distinguished from each other solely on the basis of morphological criteria.

Since these two species fundamentally differ in the manner in which they attack hair *in vitro*, this property can be used as a diagnostic aid, when correlated with their morphologic characteristics as developed on a variety of media.

T. mentagrophytes radially penetrates hair segments immersed in water forming wedge-shaped perforations. *T. rubrum* does not perforate hair.

The method of determining the ability or inability of *T. mentagrophytes* or *T. rubrum* to perforate hair and the use of this test in identifying those species are described.

Acknowledgment

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BLASTOMYCES CEROLYTICA (sp. n.) AND ITS RELATION TO COCCIDIOIDES.

by

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(2.5.1955)

In 1949 (1) we mentioned the discovery of a new species of yeast-like fungus. Being there a symbiont, it was isolated from mycetoma of certain Pyrralides and Cercopides. Its polymorphism and evolutional cycle show interesting relation to Coccidioides. We give a temporary designation of the fungus: *Blastomyces cerolytica* (or *B.c.* in text).

Isolation and cultivation

The first cultures in modified Richard solution were obtained from intestinal mycetomes of Flata marginella. They were cottony with pink colored thallus. On different media and under different pH concentrations it showed four macroscopically different types. They are:

1. Yeast-like, rough or smooth growth (Y, R or S)
2. Cottony with or without ivory colonies (filamentous "F")
3. Submerged filamentous ("F" submerged)
4. Gypsoid, floury colonies with or without pigment.

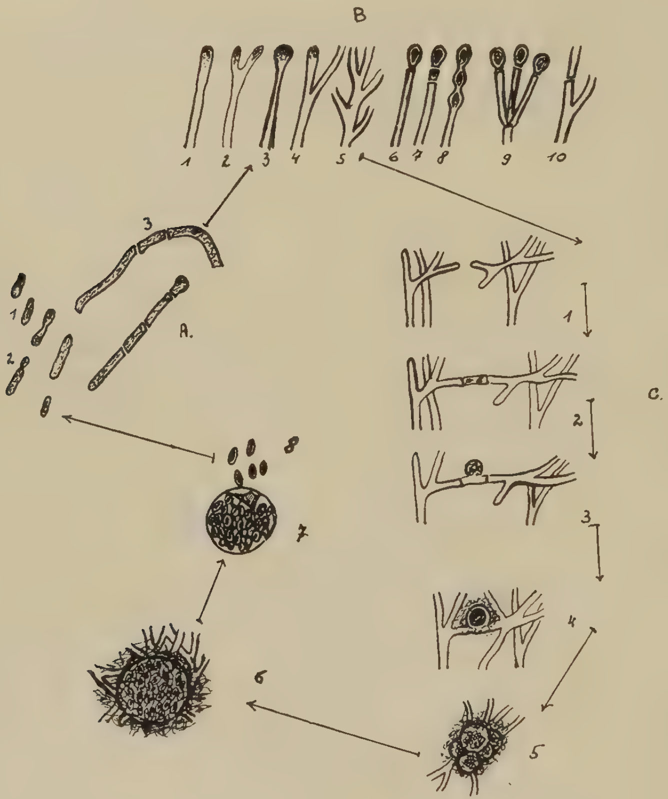
We found also intermediate forms among the above mentioned types on different media.

The yeast (Y) form has creamy R or S colonies, adherent and compact with thick membraneous aspect, segmented, at the same time encircled with filamentous areola. The colour is ivory or gray. By the time the R form has begun the formation of a fine mycelium, the colony acquires a cottony aspect. The cottony form is transformed after two weeks into a gypsoid powdered surface, resembling very much *Sabouraudites lanosus*. In very acid media we can obtain submerged filamentous growth which never returns to the yeast form (F does not return to Y form). The gypsoid type can preserve its vitality for months even in absolutely dry media.

In glabrous colonies we find yeast cells in different stages of budding. Budding is always unipolar, resembling partly the Hansenulla genus. Their cytoplasm has a net like aspect with numerous vacuoles, and chromatophil granules. It shows Gram positive granular structure. HUNTON and LEGROUX staining shows the existence of a thin capsule. In getting old, the cultures show elongated forms 3—5 times their original length. Later they form a septate mycelium. The Y—F phase is usually very short, depending on the pH concentrations and composition of the media. At times the granulation disappears but mycelium remains still septate and resembles a ribbon of cellophane. It is difficult to stain it. We observe the disappearance of the septa, and the formation of aerial mycelium. These are fine prolongations of the thallus inclined horizontally at angles of 90°, and for this reason, it gives a hemispherical aspect to the colony. The hyphae can be round (like the head of a match), bicephal or showing pinhead form. We found aleurospores as soon as aerial mycelium starts becoming twisted. At the same time the cottony colony changes its aspect to the gypsoid one. In older cultures we observed formation of the large apical chlamydospores. They are iodophilic (Gram positive). In the compact mass of infinite anastomosis of mycelia we found entangled large number of cysts of different sizes, single or in groups. These cysts are interwoven with the plectenchyme on the thallus making them inseparable. In the cysts we distinguish a basophil and Gram positive mass, encircled by a thin chromatophobe layer. The cyst becomes polynuclear. Then, this mass will be segmented in tetrades, giving birth after an other division to mononuclear sporiform bodies. The cover of the cyst bursts after ripping, as occurs in *Coccidioides immitis*, and an extrusion of the spores takes place. The spores are oval, the nuclei are acid fast and iodophil, the cytoplasm is chromophobe, and the membrane unstained. The empty cyst (after bursting) shows a triangular tear. The extruded spores become elongated and take yeast form, or directly develop into mycelium. The plectenchym is always purple coloured, but we observed also some spores of the same colour. There is a certain resemblance between cysts of *Blastomyces cerolytica* and giant cysts (asci) of *Thelebolus stercoreus*.

Organs of fructification

We start with the quiescent polynuclear cysts. Normally there is progressive development, the nuclei are collected progressively in the cyst, and organised into spores which will extrude after maturation of the cyst. The spores become elongated and begin budding. Elongation gradually continues to the extent that we can consider it as a septate mycelium. Aerial mycelium is formed later. The organs of fructification are aleurospores and chlamydospores. The thallus forms plectenchymatic fertile stroma, which can be considered as a pseudohymenium. In this thallus spherical globules arise without any ostiole, covered with fine peridium. These spherical bodies are polynuclear cysts in which the endospores are formed — further organs of fructification of *B:c.*



Genetic Analysis

It was found that *Blastomyces cerolyt.* is bipolar. It is heterothallic isogame. The pseudohymenium of *B.c.* is formed of plectenchymatic stroma in which well defined ascogenic fertile filaments develop after anastomosis between the thalli of opposite sexes. The cysts (asci) are dispersed more or less regularly, single or in groups in the pseudohymenium without being reunited in a separated hymenium or in proper perithecia. This mode of reproduction permits *B.c.* life on a support and in a constantly humid atmosphere.

The diploid form of *B.c.* is represented only by hooks on the ascogenic filaments up to the third division of the mother spores, at what time meiosis and segregation of sexes become manifest. All other forms of *B.c.* are haplobiontic, therefore, in this case, diplont develops as a parasite on haplobiontic thallus. The yeast form is strictly haplobiontic. The duration of dicaryon is determined by physiological method by presence or absence of certain factors of biochemical nature. These factors cannot be designated as growth factors because the growth is independent from them. They influence only the duration of dicaryophase. These factors we designated as CARYOGAMY FACTORS.

Inoculating the spores of a cyst we get two groups of haplonts of different sexes. These strains are the basis for determination of Mendelian factors in unknown strains. This operation entails:

1. Inoculation of spores in monosporic cultures.
2. Reinoculation and isolation of haplonts arising from germination of spores.
3. Examination of these haplonts two by two and in all possible combinations.
4. Establishment of tests based on result of examination. These tests represent the Mendelian factors.
5. Conservation of tests as a means of comparison for other determinations.

We obtained monosporic culture in cultivating cysts in highly concentrated monosaccharide media (40 % glycose). The osmotic pressure in the cysts is always higher than that of the medium in which they are submerged, in other words, they are hypertonic in comparison with the medium. Thus, pressure can reach or even exceed 300 atmospheres, and the LAPICQUE (4, 5) phenomenon, named epictese, appears to which bursting of the ascus is ascribed. The spores obtained after bursting of cysts are well separated and dispersed. After centrifugation the sediment was diluted and inoculated in solid media in Petri dishes of large diameter. The colonies, thus obtained, are monosporic and haplobiontic. These colonies ought to be reinoculated in Gorodkova agar and transferred to test medium of Buller or Vandendries.

The perfect reproductive form of *B.c.* (asci) cannot be obtained without the presence of Caryogamy factors in the medium. Similar factors are known in certain Pyrenomycetes as HEALD-POOL phenomenon (6). The caryogamy factors can be obtained from extracts of pollen grains, waxes, potatoes, egg-yolk, blood, podophillium resine, macerated intestines of certain insects, and cultures of some Aspergilli. Only the presence of these factors in the medium permit the copulation of thalli to be fertile. If not, the dicaryophase becomes protracted without copulation. Afterwards the dicaryon is desintegrating and it is eliminated through formation of false hooks. Therefore the haplon (+) and the haplont (—) with caryogamy factors give rise to stable diplobiontic forms with production of cysts, with segregated endospores.

Biochemical properties

Blastomyces cerolytica grows in pH concentration between 3.4—5.2. It can develop even outside these limits but with difficulty and with long retardation. Above pH 5.2 we obtain glabrous smooth colonies which never produce an aerial mycelium. Below 3.4 pH the colonies are floury, very dry and fragile. Reinoculation rarely succeeds. *Blastomyces cerolytica* need good aeration for all stages and all forms. Oxygen consumption is very high. The fungus is not thermophile, it develops at room temperature, and even at low temperatures (10° C) but a bit delayed. Infrared rays favour development of mycelium, ultraviolet rays delay transformation from one form to another. The fermentation tests are carried out with the GUERRA (8) method, with yeast form. We prepare a thick emulsion of *Blastomyces cer.* yeast form and test with different carbohydrates in Guerra tube's.

TABLE I

Fermentation table

Sugar	Results	Reading time
Glycose	+ +	24 hrs
Saccharose	+	4 days
Maltose	+	7-days
Lactose	—	—
Raffinose	+	15 days
Mannitol	+ + +	three days

According to this result *Blastomyces cerolytica* is a zymatic, malto-saccharasic yeast.

Auxanograms were carried out according to BEIJERINCKS (8) method. It showed that *Blastomyces cer.* consumed: glycose, saccharose, lactose, galactose, maltose, raffinose, mannitol, glycerol, paraffinol, waxes, naphta-petrol; not consumed: ethanol, methanol, aceton, acetic acid, amyl alcohol, benzoic or salicylic acid. Tests with nitrogenous substances show that it consumes: Ammonium salts, peptone, glycol, little urea; not consumed are: nitrates, nitrites, rhodanides, cyanides, iso-cyanides and histidine. Traces of iron are necessary for formation of pigment. The color is pale rose. Only in the presence of caryogamy factor appeared the dark pink pigment on the basal side of thallus, and purplish color appeared in the aerial mycelium. The pigment is hardly water soluble, and hardly separable from the thallus. Only by cathionic and non ionic wetting agents (detergents) can we separate and extract this pigment. Further particularities about isolation of pigment see elsewhere (MUFTIC 3). Among the enzymes *Blastomyces cerolytica* produces: maltase, saccharase, glycolase, protease (gelatine liquefied) and cerase (waxes and paraffines decomposing cyclophorase-oxidase). *Blastomyces cerolityca* is a rare fungus utilising wax as carbohydrate source. For that reason it is termed: *Cerolytica* (wax decomposing).

Symbiotic role of Blastomyces cer. in different Homoptera

As mentioned before, *Blastomyces cerolytica* was isolated from mycetomes of certain Cercopides, Zycades and Pyrralides. There it actively participates in digestion of waxes. Different authors (LAHNAM 20, STEINHAUS 21, PEKLO 22, TOTH 23, and SMITH 24) have described yeast like fungi (*Saccharomyces*, *Aphidomyces* etc.) which are the symbiont in mycetomes of different Aphides. Their role and indispensability for a normal digestion are well established and described (HAUPT 15, MUFTIC 1, SCHWARTZ 16).

10 % potassium iodid, and 5 % undecylenic acid were completely ineffective against *B.c.* suspension of yeast form; injected intraperitoneally into rats it did no harm. Observation period was about 7 months. In guinea pigs injection of chlamydospore suspension caused enlargement of regional lymph nodes in two weeks without further development of a disease. These nodes were inoculated in Gorodkova agar, but did not give rise to colonies.

Systematic position

There is a number of yeast like fungi which occur in yeast phase in vivo and under special conditions, but in vitro they usually grow in a filamentous phase when incubated at room temperature. The environmental conditions in animal tissues restrict mycelial development. It is possible that Y phase and F phase formation is elicited by biochemical factors which are mutually exclusive and that the transition from one phase to the other is governed by rather special nutritional and temperature requirements. Similar conditions are necessary for the production of the dicaryon phase and of the diplont. The great morphologic resemblance between *Blastomyces cerolytica* and *Coccidioides immitis* (see the table No I) can give some idea about position of *Coccidioides*, under special morphologic or genetic conditions. It is quite clear that *Blastomyces cerolytica* belongs to a transitory phase between Endomycetales and Taphrinales. The question of systematic position of *Coccidioides immitis* has a long history. LANGERON (7, 8) proposed its approachment to Saprolegniales, Peronosporales, Chytridiales etc. A. FISH (17) ascribed it to Protomycetes. DA FONSECA (11) referred it to Hemiascomycetes (see *Protomyces macrosporus* and *P. pachydermus*). M. MOORE (10) and C. W. DODGE (9) believed that

Coccidioides can be attached to Endomycetales (opinion which Langeron opposed categorically). Gauman (18) assigned them to Taphrinales. L. Bogliolo and J. Areira Neves (19), according to Vuillemin (13) classification termed *Coccidioides* an *Aleurisma.*

We give the following diagnosis for the *Blastomyces cerolytica* (sp. n.): In poor synthetic media it produces glabrous colonies which after a week become cottony with aboundant aleurospores, simple and apical. The mycelium is rectangular, branched like fine chromophobe, hyaline ribbons. In rich natural media there are plenty or chlamydospores, intercalar of apical. The mycelium is heterothallic isogame and give many anastomosis, but dicaryophase will be reached only if the medium contains the caryogamy factors. The fertile mycelium (diplontic) produces the giant cysts (asci). The asci at the beginning show the presence of the tetrades, which, later on, are differentiated in ascospores. After bursting of the asci the spores, sexually segregated, give rise either to yeast forms or filamentous growth. There is no special hymenium. The number of ascospores are many hundreds.

Table II.

The characteristics	Coccidioides immitis	Blastomyces cerolytica
Aleurospore	2—4μ φ	2—μ φ
Chlamydospore	5—11μ φ	3—12μ φ
Asci (cysts)	4—80μ φ	4—150μ φ
Ascospore	2.5μ φ	2—4μ φ
Sabouraud agar	Creamy, ivory colonies, with concentric circles	Creamy, ivory colonies with irregular segments.
Gelatine	Softened	Liquefaction
Potatoes	Cottony with many chlamydospores	Gypsoid, with pink pigment
Yeast phase	Unknown	Haplobiontic yeast
Heterothallism	Unknown	Isogame
Inoculation	Successful	Unsuccessful

Summary

In 1949 I discovered a yeast like fungus which was termed *Blastomyces cerolytica*. During the essay for exact determination of this fungus, it was found that it resembles *Coccidioides*. Its yeast-like form is zymatic, malto-saccharasic. As organs of fructification aleurospores and chlamydospores were found, as well as asci containing hundreds af ascospores. It shows isogame heterothallism. It belongs to Taphrinales. A comparison between characteristics of *Coccidioides immitis* and *Blastomyces cerolytica* is given. The diagnosis of this fungus concludes the investigation.

Resumé

En 1949 nous avons découvert un champignon levuriforme, lequel nous avons nommé *Blastomyces cerolytique*. Il nous a paru d'être très semblable au genre *Coccidioides*. Son état levuriforme se comporte comme une levure zymatique et malto-saccharasique. Les formes asexuelles sont des aleurospores et des chlamydospores et les formes sexuelles des asques géants remplis de centaines d'ascospores. Il paraît être isogame hétérothallique. Il appartient à des Taphrinales. Une comparaison était mise en vue des caractéristiques de *Coccidioides immitis* et de *Blastomyces cerolytique*. Nous donnons aussi une diagnose pour ce champignon.

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PNEUMOMICOSI CRONICA (Aspergillosi?) CON GENERALIZZAZIONE PLURIVISCERALE

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(1.10.1954)

Premessa

Con la presente nota illustriamo un caso di micosi umana a decorso cronico, a carattere granulomatoso produttivo neoplastiforme con interessamento pluriviscerale, che ha presentato un quadro clinico e anatomopatologico assolutamente eccezionale.

Appunto per questo suo aspetto insolito e per un complesso di circostanze fortuite la giusta diagnosi fu posta solo in sede di esame istologico, quando era troppo tardi per una esatta identificazione del micete attraverso uno studio colturale e biologico. Tuttavia riteniamo ugualmente interessante la pubblicazione della nostra osservazione.

Descrizione del Caso

Riassunto della storia anamnestico-clinica.

Si tratta di un uomo, nato nel 1903. Nel 1930 assunto come infermiere di fatica in un Sanatorio e sottoposto prima di prendere servizio e poi ogni anno, a visite mediche e controlli RX del torace, veniva sempre giudicato godere ottima salute fino al 1939. In questa epoca il paziente presentò febbricole serotine, tosse con abbondante espettorato (Koch negativo); obbiettivamente vennero riscontrati all'ascoltazione del torace gruppi di fini bolle in sede sopraclaveare destra.

La lastra del torace (fig. 1) mise in evidenza notevole velatura di tutto il lobo superiore del polmone destro con piccole zone di iperdiafania e microaddensamenti. Dopo circa venti giorni la sintomatologia clinica scomparve ed il paziente riprese il lavoro godendo ottima salute.

Nel 1946 sottoposto a controllo RX del torace, la lastra (fig. 2) mise in evidenza segni di opacità polmonare, a partenza para-ilo-mediastinica superiore destra, con risparmio della parte apicale periferica del lobo superiore, leggero spostamento a destra della trachea; negativo l'esame obbiettivo.

Tale zona di opacità radiologica si è andata progressivamente estendendo fino ad occupare tutto il lobo superiore del polmone destro (fig. 3 — anno 1950).

Le condizioni generali del paziente si mantenevano buone; accurati periodi di osservazione in Sanatorio con controlli batteriologici dell'espettorato, non riuscirono mai a documentare la sospettata patogenesi specifica delle alterazioni del parenchima polmonare.

Fino al 26—9—1953 il paziente continuò a svolgere la propria mansione quando apparvero tosse con catarro, febbre dispnea, seguiti dopo pochi giorni, da violenti dolori localizzati all'addome destro e rapido decadimento delle condizioni generali. Ricoverato all'Ospedale di Niguarda i vari esami clinici, radiologici, di laboratorio eseguiti misero in evidenza:

a) sistema nervoso: psiche parzialmente obnubilata, con brevi periodi di perdita di conoscenza, sensorio solo in parte conservato, emiparesi destra, afasia motoria, segni radiologici di ipertensione cranica. Reazioni di Wassermann e colloidali per la lue: negative.
b) torace: accentuata dispnea con tosse e scarso catarro; conferma (fig. 4) dei dati radiologici precedenti con dimostrazione stratigrafica di occlusione completa del bronco lobare superiore destro; espettorato Koch negativo.
c) addome: dolente al quadrante sup. destro; la colecistografia dimostrava una colecisti normale; la pielografia discendente evidenziava che il rene destro non era iniettabile; nelle urine si repertava velo di albumina con rare emazie e leucociti nel sedimento; azotemia 0,37; glicemia 0,84.

Mentre si attendeva di procedere ad altri esami per poter concretare la diagnosi, il paziente si dimise di propria volontà contro il parere dei Sanitari. Ricoverato in gravissime condizioni nel Sanatorio di Garbagnate decedeva pochi giorni dopo.

Reperto necroscopico (prot. No. 2117 in data 27.XI.1953).

R. Giovanni

Cadavere di sesso maschile, età apparente 50—55, normotipo, condizioni generali di trofismo modicamente scadute.

Per divieto dei parenti non si apre il cranio.

All'apertura del cavo toracico si osserva il cavo pleurico sinistro libero da versamenti e aderenze. Il cavo pleurico destro è completamente obliterato: nella parte inferiore le aderenze con carattere di membrane fibrose sono, sia pure con certa difficoltà, vincibili. Nella parte superiore i foglietti pleurici sono fusi in una spessa cotenna che infiltra anche le parti molli extrapleuriche della parete

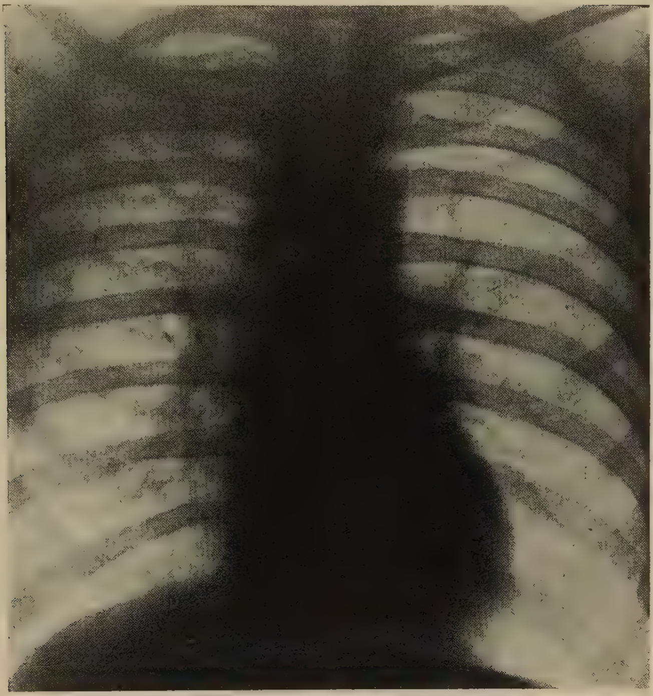

Figure 1. Radiografia torace (1939).

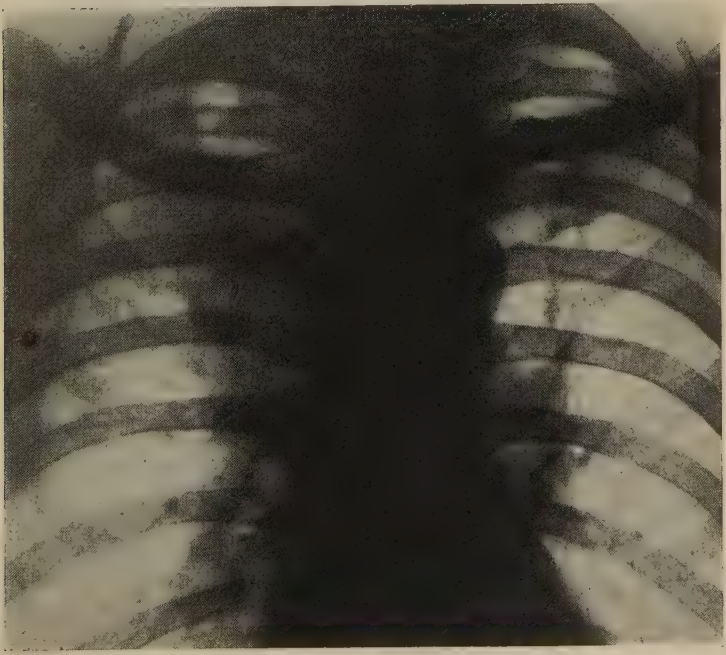

Figure 2. Radiografia torace (1946).

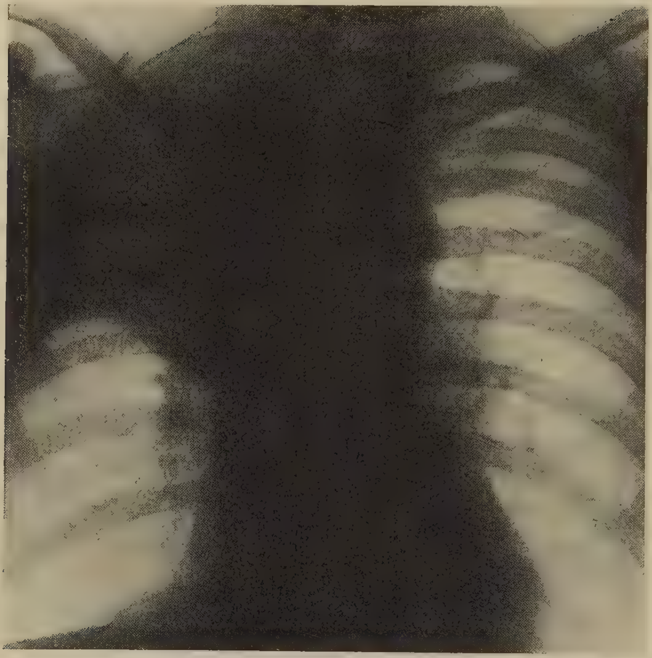

Figure 3. Radiografia torace (1950).

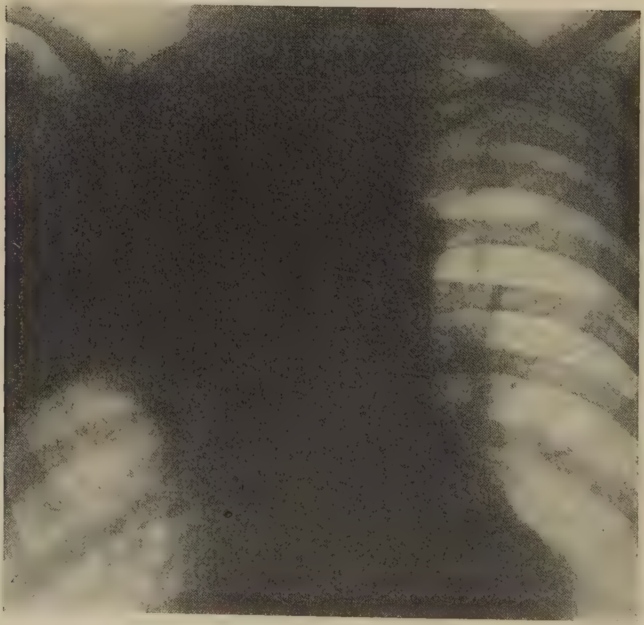

Figure 4. Radiografia torace (1953).

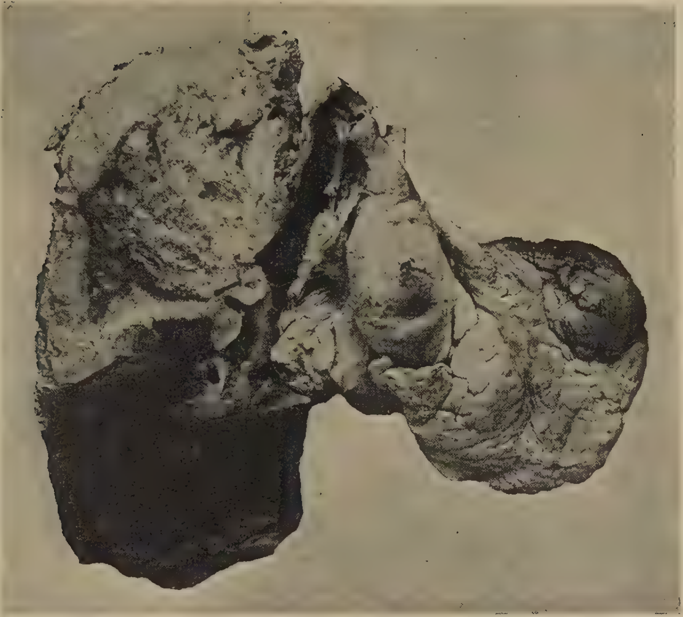

Figure 5. Polmone ds., cuore e organi del mediastino. Osservare la massa neoplastiforme del lobo sup. ds. e quella cardiaca sulla faccia ant. del ventricolo sin. e sul bordo ottuso.

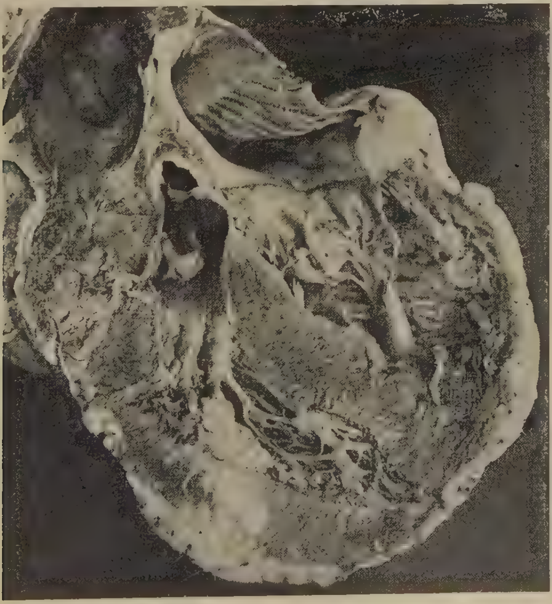

Figure 6. Infiltrazione granulomatosa della parete del ventricolo sin. (a sinistra dell'osservatore).

toracica cosicchè si è costretti a ricorrere al coltello per l'estrazione dell'organo. Il cavo pericardico è libero da aderenze e versamenti. Il tratto intrapericardico di alcuni grossi vasi venosi (vene polmonari sin. e vena cava sup.) e dell'arteria polmonare destra appare avvolto da un mantello di tessuto bianco, lardaceo: una massa vegetante con gli stessi caratteri, a superficie bozzoluta, poco rilevante, si osserva sulla faccia anteriore del ventricolo sinistro e sul bordo ottuso del cuore.

Cavo peritoneale: visceri in situ, fegato lievemente debordante dall'arcata costale, non versamento. Sierosa sottile, liscia e lucente.

Esame dei visceri:

Cuore (fig. 5—6): presenta volume modestamente aumentato. Si osserva sulla faccia anteriore del ventricolo sin. e sul bordo ottuso una vegetazione poco rilevata a larga base di impianto, del diametro di cm. 7—8 con superficie grossolanamente mammellonata, liscia e lucente di consistenza dura. Al taglio si osserva che essa è costituita da un tessuto uniforme, bianco, lardaceo, di consistenza assai spiccata che penetra negli strati muscolari superficiali e si spinge in qualche punto sino alla superficie endocardica.

Un nodo di aspetto analogo, della forma e dimensioni di un pisello, si osserva sul tessuto subepicardico in corrispondenza del punto medio del solco coronarico posteriore. Un mantello di tessuto con gli stessi caratteri avvolge il tronco del ramo destro dell'arteria polmonare, le vene destre ed il moncone prossimale della cava superiore.

Polmone destro (fig. 5): si presenta ingrandito. La pleura forma intorno al lobo superiore una sorte di spessa capsula fibro-lardacea, mentre intorno al lobo inf. e medio appare modestamente ispessita. All'ilo si osserva una ganga istiale che avvolge gli organi vascolari ed i linfonodi della regione (pur essi ingranditi, ma ancora riconoscibili) e salda assieme pleura mediastinica destra e organi del mediastino, in particolare fra questi la vena cava ascendente.

Al taglio si rileva che tutto il lobo superiore è sostituito da un tessuto con gli stessi caratteri, ma screziato da tratti con colore ardesiaco. Esso è più addensato verso l'ilo; il bronco superiore appare appena accennato al suo inizio, per scomparire subito nel tessuto neoformato che lo oblitera totalmente. Alla periferia si osserva una banda di tessuto lardaceo, spesso da 2 a 4 cm. che si continua anche in corrispondenza dell'interlobo. In una sezione trasversale alquanto posteriore si osservano due piccole cavità contenenti un materiale similpurulento.

In corrispondenza del lobo inf., in regione parailare, il bronco inf. appare avvolto da un nodo di tessuto con gli stessi caratteri. Nel rimanente ambito parenchimale note di stasi ematica cronica.

Polmone sinistro: non reperti patologici tranne un modesto stato di stasi ematica nelle regioni inf. e posteriori dell'organo.

Mediastino: come si è detto la pleura mediastinica ds. del lobo sup. aderisce agli organi del mediastino superiore che appare parzialmente infiltrato dal tessuto neoformato. In particolare il tronco della cava sup. ne appare totalmente avvolto. Nulla di particolare alla trachea e all'esofago. I linfonodi peritracheali, della biforcazione ed ilari sono indenni; quelli dell'ilo polmonare destro (ingranditi e di colorito screziato bianco e grigio) sono avvolti dalla ganga del tessuto neoformato.

Organi del collo: n.d.n.

Organi addominali: milza un poco tumefatta, molle; perisplenio liscio. Al taglio, la polpa è modestamente aumentata, di colorito rosso vinoso, facilmente asportabile dal coltello.

Nulla di patologico si rileva a carico dello stomaco e dell'intestino.

Il *fegato* è modestamente ingrandito con periepate liscio; nel parenchima si osservano note diffuse, di grado modesto, di stasi ematica cronica.

Nella grande ala presso il margine acuto, al di sopra del letto colecistico, ma ovunque circondato da tessuto epatico si nota la presenza di un unico nodo, della grandezza di una grossa noce, a confini policiclici e netti verso il circostante parenchima, nodo formato da tessuto bianco, di particolare consistenza, uniforme di aspetto, simile in tutto a quello repertato nel polmone destro e nel cuore.

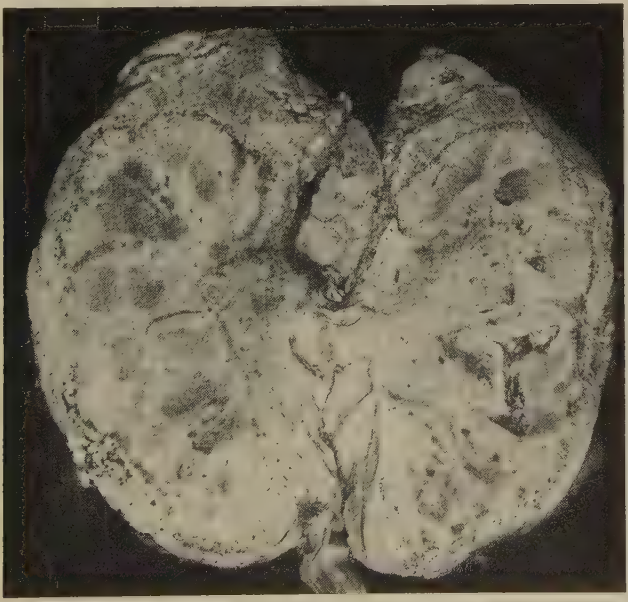

Figure 7. Infiltrazione massiva e diffusa del rene ds. (metà della grandezza del reale) e della capsula celluloadiposa del rene.

Tutta la *loggia renale* destra è occupata da una massa di tessuto duro, compatto che ingloba il rene ed il surrene (fig. 7), nonchè i vasi dell'ilo ed i linfonodi paraaortici di destra. Questa massa deve essere asportata in toto. Condotto un taglio frontale passante per il maggior piano del rene si osserva come questo sia ingrandito (l'asse longitudinale di cm. 15 è circa due volte quello controlaterale) pur conservando la forma normale: è ancora visibile una traccia di una normale struttura anatomica con possibilità di distinguere corticale, midollare e calici. Questi sono peraltro deformati, stretti e allungati. Alla periferia il rene fa corpo con una massa di tessuto biancastro, a limiti irregolari verso il connettivo cellulare della loggia renale che in gran parte occupa e che forma al rene come una spessa capsula di circa 2 cm. di spessore. Verso il polo superiore inglobato in essa si osserva il surrene destro peraltro non infiltrato nè ingrandito. Verso l'ilo detto tessuto avvolge il bacinetto ed il tratto iniziale dell'uretere ed alcuni linfonodi paraaortici (fig. 8), ingranditi come noccioline, pur essi infiltrati ma ancora riconoscibili nella ganga del tessuto lardaceo.

Il *rene sin.* si presenta di volume normale, ben scapsulabile, con superficie liscia: al taglio, nel parenchima corticale, si osservano quattro nodi della grandezza da un pisello a una ciliegia, a limiti netti di tessuto biancastro, simili a metastasi cancerigne. *Surrene sin.:* n.d.p.

Nel terzo inf. dell'*uretere destro* un tratto di circa 10 cm. di lunghezza appare infiltrato dal tessuto patologico. Il lume è dilatato, la superficie interna irregolare per rilievi ora a forma di polipo ora di bottoni che infiltrano anche la tunica esterna muscolare ed avventiziale dell'organo.

Nulla di patologico all'uretere sin., alla vescica, alla prostata. *Organi genitali* indenni.

Considerazioni Diagnostiche Macroscopiche

Il quadro anatomico apparve subito insolito, anzi eccezionale.

Il carattere altamente produttivo del tessuto patologico, il suo aspetto biancastro, lardaceo, denso, duro; l'aspetto nodulare multiplo di alcune sedi (fegato, rene sin.) indirizzavano verso una forma neoplastica. Peraltro strano appariva il comportamento della lesione in altre sedi. La diffusione centrifuga nel lobo sup., la completa obliterazione del bronco sup. suggerivano la primitività pleuro-polmonare della supposta neoplasia ed in sede di discussione epicritica si poteva pensare — concordemente alla diagnosi clinica — ad un ca. bronco-polmonare insorto in terreno di vecchia forma tbc produttiva. Ma la tendenza alla diffusione sistematica alle sierose (pleura, pericardio), la tendenza ad infiltrare i cellulari lassi mediastinico e pararenale destro, lo scarso interessamento dei linfonodi ilari e peritracheali, infine la infiltrazione diffusa, sistematica, dissociante del rene destro, che conservava ancora il disegno anatomico generale, con un comportamento analogo alle infiltrazioni

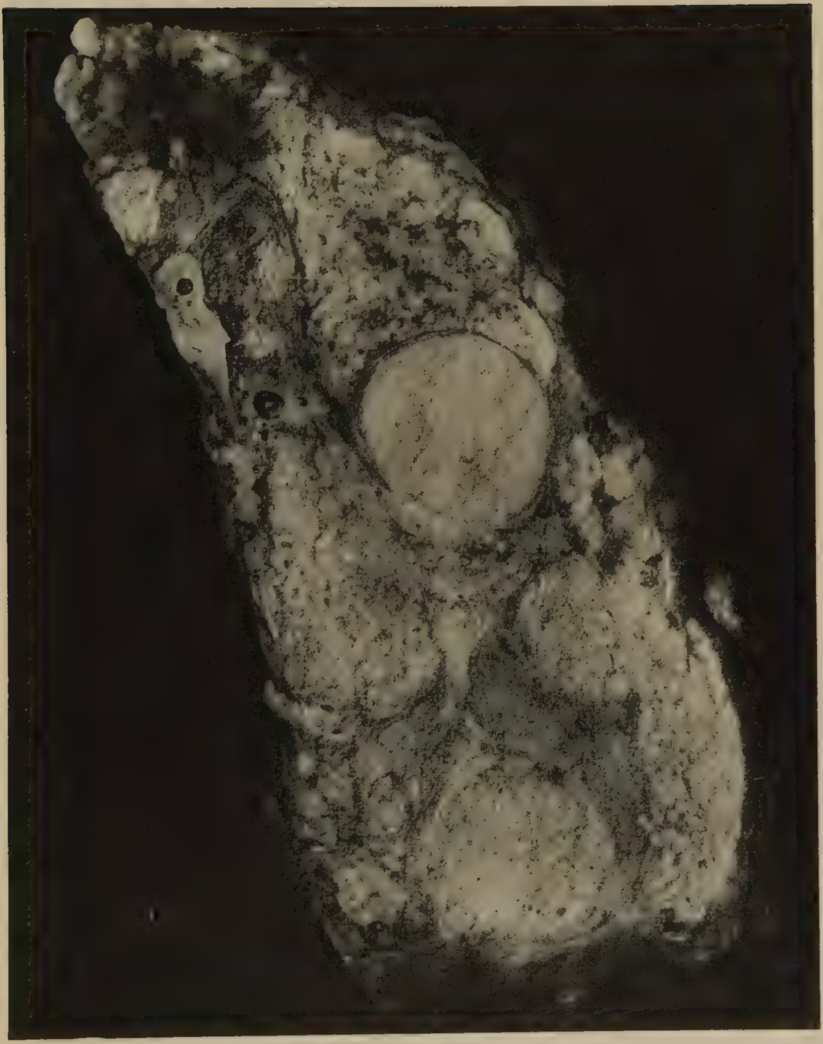

Figure 8. Massa granulomatosa paraaortica addominale ds. interessante linfonodi e cellulare (ingrandito circa 6 diametri).

leucemiche, lasciavano qualche perplessità. Tuttavia in sede di necroscopia si concluse come probabile la natura neoplastica delle lesioni. La natura infiammatoria granulomatosa venne respinta per l'intensità della neoformazione di tessuto, per gli aspetti similmetastatici ed, infine, per gli stessi caratteri fisici del tessuto neoformato. Anche l'eventualità di granuloma micotico fu rapidamente presa in considerazione per essere subito respinta, non essendo noto nell'uomo forme micotiche che possano dare il quadro anatomico osservato.

Purtroppo questo deciso orientamento negativo nei confronti della micosi portò all'omissione di ricerche colturali, biologiche in questo senso, togliendo così la possibilità di una più precisa diagnosi. Furono invece praticate ricerche per il bacillo di Koch sia microscopiche che colturali e biologiche su materiale prelevato sulle cavernule polmonari e su materiale pseudoneoplastico triturato, allo scopo di documentare la coesistenza di una forma tubercolare che avrebbe nel tempo preceduta quella neoplastica. Gli esami diedero tutti esito negativo.

Reperti Istologici

Le lesioni istologiche nei vari organi interessati presentano sostanzialmente gli stessi caratteri pur tuttavia offrendo qualche particolarità diversa da sede a sede.

Si tratta di un tessuto infiammatorio granulomatoso cronico (fig. 9) costituito dall'aggregazione di una miriade di micronoduli elementari; il suo aspetto è di una grande uniformità.

Il micronodulo (fig. 10) di dimensioni relativamente costanti fra i 100—300 micron presenta al centro una o più cellule giganti multinucleate con vasto citoplasma di forma irregolare e variabile, simili spesso ai grandi simplasti dei tessuti granulomatosi da corpo estraneo.

La caratteristica fondamentale di questi elementi cellulari è data dalla presenza nel loro citoplasma di cellule fungine, che saranno dettagliatamente descritte più avanti. Esse si trovano in numero vario da 1 a 10 elementi ed il citoplasma delle cellule giganti forma attorno a loro, quando sono numerose, come un sottile camice plasmatico. I nuclei rotondi, di medie dimensioni in numero vario da 4 a 10 e più, sono in genere respinti alla periferia. La cellula o le cellule giganti sono circondate da numerosi elementi di tipo istioide, con morfologia varia, di solito poligonale, più spesso con citoplasma chiaro e nucleo vescicoloso pallido, assai simile alle cellule epitelioidi del granuloma tubercolare. Anche in esse si possono talvolta osservare elementi fungini in numero di 1 o 2 al massimo. Oltre ai conponenti citologici descritti, abbastanza numerose sono le cellule linfoidi con la loro caratteristica morfologia e, più raramente, plasmacellule uni o binucleate. Polimorfonucleati neutrofili ed eosinofili sono piuttosto rari.

Numerosi sono invece i fibroblasti, stellati od allungati; si possono

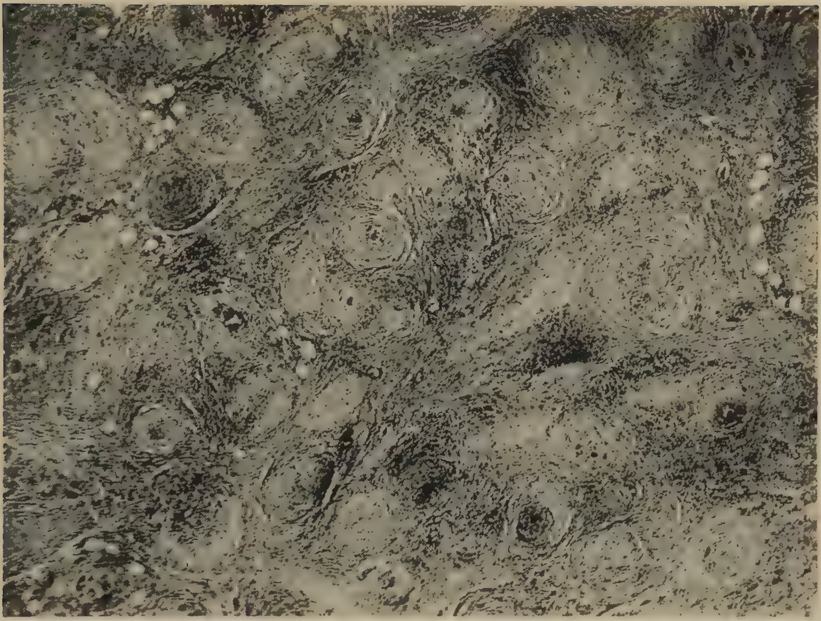

Figure 9. Color. emat. - eosina - × 31-Pleura parietale ds. Visione a piccolo ingrandimento del tessuto granulomatoso risultante dalla giustapposizione di numerosi piccoli noduli elementari. Osservare la grande uniformità delle strutture presenti in tutte le localizzazioni.

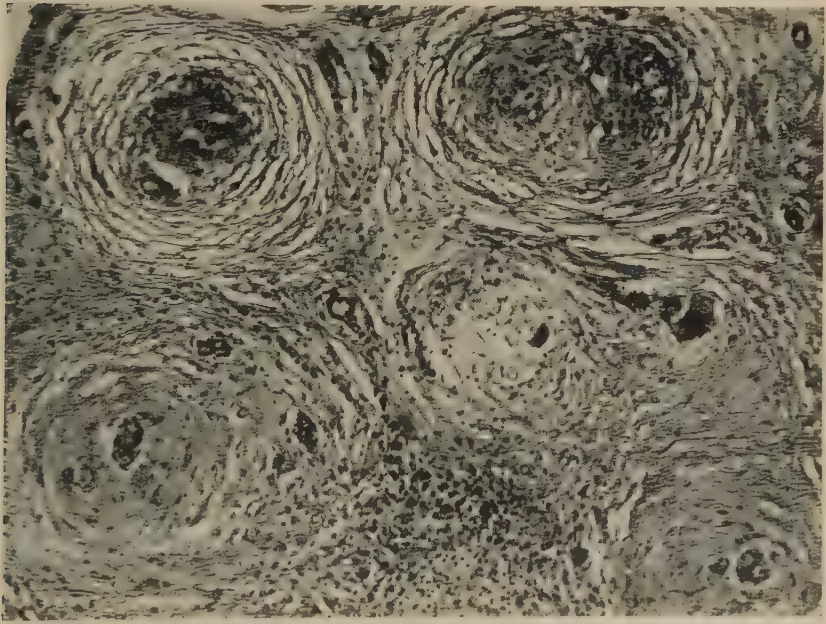

Figure 10. Color. emat. - eosina - × 107-Massa granulomatosa cardiaca. Quattro microgranuli, alcuni dei quali in avanzata fase sclerocicatriziale.

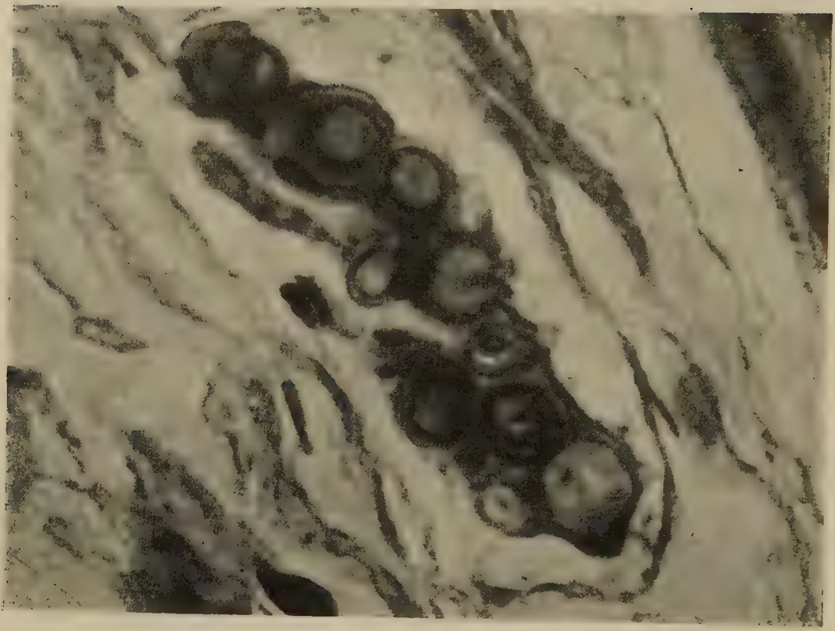

Figure 11. Polmone: color.: m.di Hotchkiss- × 3000 Clamidospore.

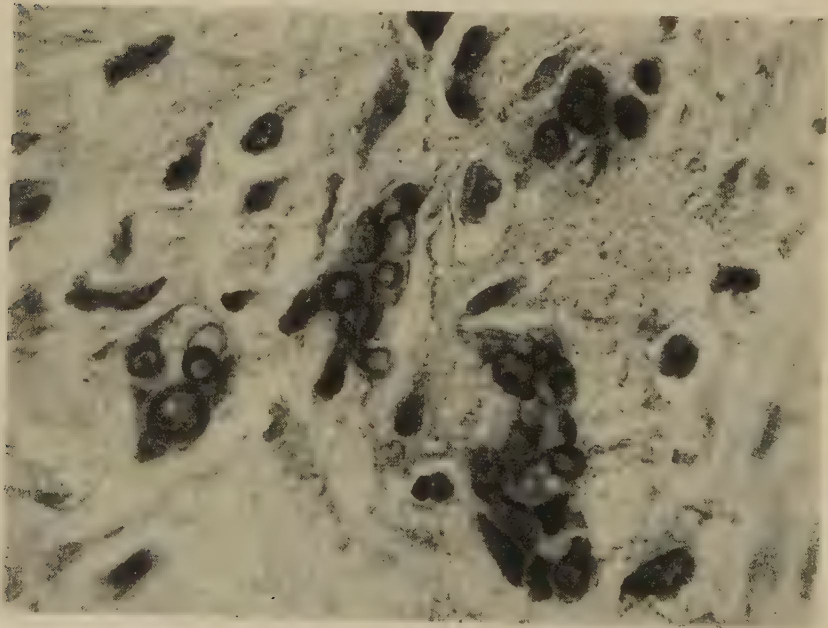

Figure 12. Polmone: color.: m.di Hotchkiss- × 975 Clamidospore catenulate.

osservare i vari gradi di passaggio fra gli elementi istiocitari indifferenziati ed i fibrociti maturi. A questo punto va detto che tutte queste cellule che circondano gli elementi giganti centrali, sono relativamente poco numerose e fitte, perchè esse giacciono in un abbondante connettivo fibrillare che presenta prevalentemente i caratteri morfologici e tintoriali del collagene maturo e più raramente, ed in aree meno estese, quelli del connettivo reticolare. Le fibre, condensate ora in spesse formazioni compatte, ora in reticoli anastomotici più lassi, presentano spesso nel loro insieme un orientamento circolare attorno al centro del micronodulo dando a questo la caratteristica forma sferica. Laddove le maglie delle fibrille sono più ampie non è raro trovare libere nella sostanza fondamentale cellule fungine. Alla loro periferia i noduli si saldano e compenetrano gli uni con gli altri pur conservando nell'insieme una distinta individualità; il tessuto presenta nel suo complesso un aspetto assai simile a certi conglomerati di tubercoli.

La vascolarizzazione è scarsissima e raramente si osserva nel micrognanuloma descritto la presenza di capillari, che, ove esistano, stanno alla periferia del nodulo stesso.

La ricchezza di connettivo neoformato, il suo alto grado di maturità, la scarsità infine della vascolarizzazione, costituiscono le basi istologiche dei caratteri fisici macroscopici: del colorito bianco, dell'aspetto lardaceo, della grande durezza del tessuto.

In linea di massima non si osservano aree fibrosiche cicatriziali pure ed estese e rarissime sono le zone di necrosi, così che il tessuto granulomatoso appare ovunque con gli stessi caratteri, presentando, come si è detto, una estrema uniformità.

Tuttavia nei vari organi si osservano variazioni dipendenti in parte da caratteristiche del substrato invaso, in parte da fattori non facilmente precisabili.

Nel parenchima polmonare il tessuto granulomatoso è talvolta intercalato da aree di strutture alveolari atelectasiche; nelle aree confinanti fra tessuto infiammatorio e tessuto polmonare il pigmento antracotico appare anche nel connettivo patologico neoformato del granuloma. Qualche area connettivale pigmentata si osserva anche nel mezzo dei grandi conglomerati. Il polmone è inoltre l'unico organo in cui si hanno focolai necrobiotici di una certa entità; in corrispondenza delle cavernule macroscopicamente descritte si osserva una necrosi caratterizzata istologicamente da un materiale finemente granulare, non strutturato, debolmente eosinofilo, uniforme con scarsissimi residui nucleari e con modestissima infiltrazione leucocitaria. Numerose si osservano in questa sede le cellule fungine, libere nei detriti necrobiotici.

Nella pleura destra lo stipamento dei micronoduli è assai marcato ed uniforme: non si osservano aree interstiziali di tessuti preesistenti conservati in sede al conglomerato granulomatoso ed i confini con il tessuto polmonare verso il lato interno, e con il tessuto connettivale — muscolare — periosteo verso la parete toracica, sono talora assai netti. Da rilevare per contro che in altre sedi il granuloma deborda dai limiti anatomici della pleura parietale per invadere, sia pure per breve tratto, i tessuti della parete toracica.

Nella zona ilare e nel mediastino il tessuto granulomatoso è irregolarmente distribuito: più intenso e sviluppato nel celluloadiposo interstiziale e perivascolare che nei linfonodi, questi pure peraltro parzialmente invasi, ma meno massivamente e globalmente; da ciò la possibilità di distinguerli macroscopicamente dal tessuto circostante.

Nell'epicardio e negli strati esterni del miocardio il tessuto granulomatoso è compatto ed uniforme, mentre negli strati più interni della parete del ventricolo esso procede in modo più discontinuo, infiltrando e dissociando i fasci muscolari; le aree miocardiche rimaste tra i gettoni del granuloma infiltrante sono però ben conservate.

Il comportamento del granuloma nel rene sinistro e nel fegato da un lato e quello del rene destro dall'altro è assai dissimile.

Nei primi due organi infatti i noduli infiammatorii osservati appaiono con struttura uniforme, senza inclusione in essi delle strutture organiche preesistenti ed i loro confini verso i parenchimi circostanti sono netti. Nel rene destro il tessuto granulomatoso infiltra tutto l'organo conservando tuttavia in molti punti le vestigia di strutture renali ed in particolare dei glomeruli e delle ramificazioni arteriose, nonchè la parete dei calici e del bacinetto, che sono ancora riconoscibili nonostante l'infiltrazione dei suoi strati esterni. Per la capsula adiposa del rene destro e per il tessuto dell'ilo renale e paraaortico si può ripetere quanto detto rispettivamente per la pleura e per il tessuto mediastinico; anche qui il primo appare massivamente infiltrato mentre nel tessuto periaortico sono invasi sia il cellulo-adiposo che i linfonodi, più intensamente il primo che i secondi.

Notevole l'assenza di invasione del surrene destro incluso nella ganga del tessuto granulomatoso perirenale. Infine nell'uretere destro il tessuto infiammatorio produttivo presenta un comportamento irregolare, ora espansivo e vegetante, ora infiltrante e dissociante le strutture dell'organo (strati muscolari).

Studio del Micete nei Tessuti

Che si tratti di un parassita fungino non vi è alcun dubbio. Il suo reperto, che possiamo dire grossolano, la sua grande abbondanza nei microgranulomi, sia entro le cellule giganti come nell'interstizio, la evidenziazione netta di ogni sua particolarità morfologica (particolarmente con il metodo Mac Manus — Hotchkis, ma anche con il metodo di Gram, e con la stessa ematossilina-eosina) sono tutti elementi che tolgono ogni dubbio circa la identificazione generica. Si tratta pertanto decisamente di un micromicete. Purtroppo nei tessuti esso si presenta con forme semplici, elementari e, mancando gli aspetti della fruttificazione o comunque forme evolute e com-

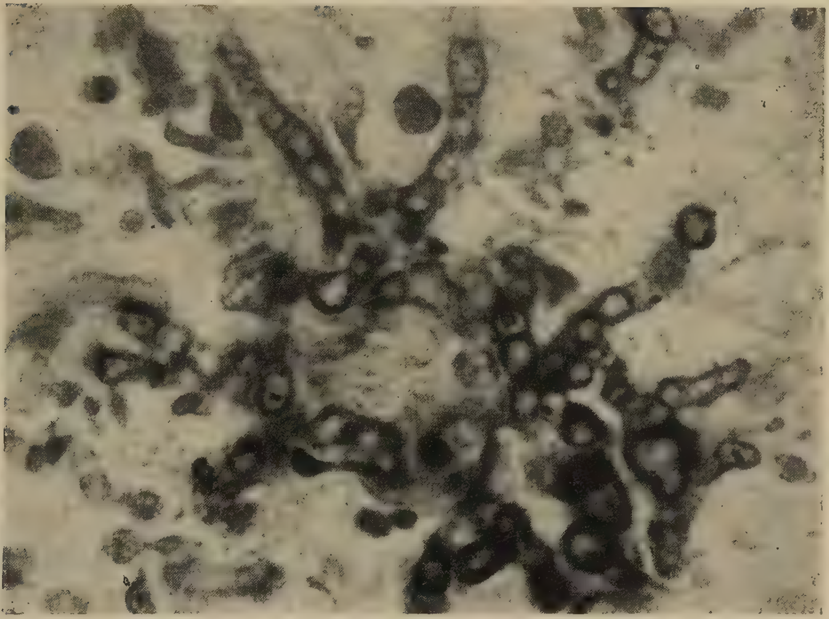

Figure 13. Polmone: color.: m.di Hotchkiss-× 1200 Catenulazione di clamidospore con gemmazione delle stesse.

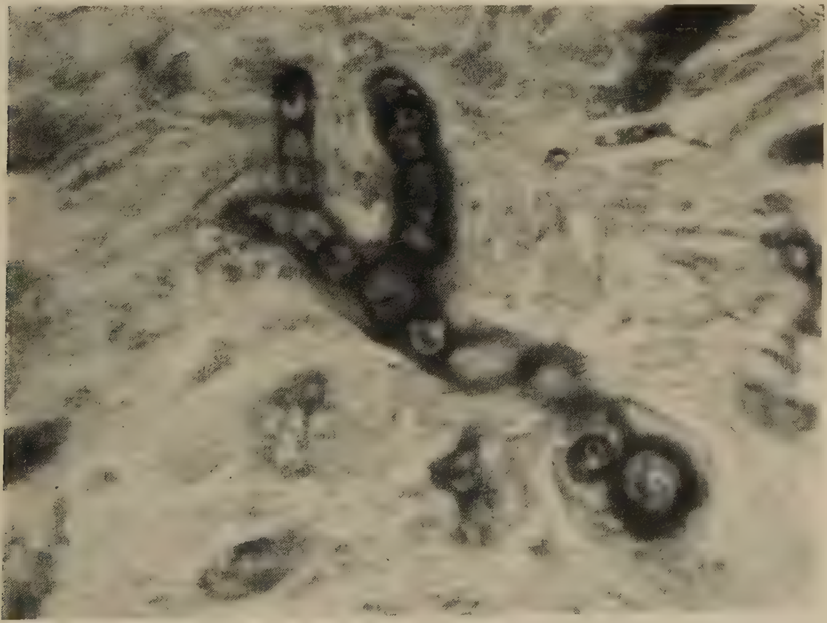

Figure 14. Polmone: color.: m.di Hotchkiss-× 1900 Id. come figura precedente a maggior ingrandimento.

plesse (quali quelle che lo stesso ha certamente presentato in natura ed avrebbe presentato nelle culture qualora fossero state fatte), non è possibile giungere ad una diagnosi micologica specifica. Ci accontenteremo pertanto di una diagnosi micologica molto generale ed approssimativa.

Il parassita si presenta per lo più in forme globose, spesso rotonde, ma anche poliedriche talora quadrangolari o rettangolari e raramente piriforme o di altro aspetto. Questi elementi possono essere del tutto isolati, più spesso sono sistemati in catena semplice e talora anche pseudoramificate.

Pressocchè tutte queste forme elementari genericamente globose hanno una grandezza notevole, raggiungendo i 5—8 micron di dimensioni e talora anche di più, variando entro questi limiti col variare delle forme. Presentano una capsula esterna ben delineata, liscia, jalina e che si colora malamente con i coloranti succitati; è lievemente rosata con l'acido perijodico ed il reattivo di Schiff. Nello interno il citoplasma presenta spesso uno strato periferico sottocapsulare nettamente positivo al Mac-Manus-Hotchkis, denunciando così una struttura ricca in polisaccaridi; esistono poi nello interno zone non positive con lo stesso metodo nelle quali si possono vedere granuli colorati con ematossilina; le forme più grosse, quelle che sembrano essere le più antiche e che talora sono al centro delle piccole colonizzazioni centrogranulomatose, sono quasi otticamente vuote; il loro citoplasma non si colora più.

Abbiamo detto che appaiono catenulate; spesso la zona di contatto fra i singoli elementi del fungo è reciprocamente appiattita come se si fosse in presenza di grossi elementi artrosporei, o di elementi miceliali molto corti e tozzi, disposti in serie ed in rapporto netto di vegetazione. Talora una forma grossa, che noi classifichiamo come una clamidospora (forma vegetativa di resistenza), stà al centro di una raggiera di elementi, come sopra descritti, che catenulandosi si dispongono come i raggi; sembra in questi casi trattarsi di una clamidospora che abbia ,,gemmato'' o che abbia ,,germinato'' elementi che si dispongono ulteriormente in catena.

Un reperto interessante ai fini della precisazione delle forme in studio è il reperto di grossi elementi grossolanamente deformi, ma che mostrano due appiattimenti della membrana in corrispondenza dei quali si attaccano due catene di elementi rettangolari e quadrangolari facendo assumere al complesso il significato di un setto miceliale che dà luogo ad una ramificazione vera e propria.

Accanto a sicure clamidospore si direbbe pertanto esistere una serie di grossi e tozzi elementi miceliali, segmenti di un micelio sterile che articolandosi danno una vegetazione ramificata.

Da ciò il rilievo di una vegetazione fungina ridotta a clamidospore isolate o catenulate, e ad una semplice ridotta e degradata vegetazione ramosa, miceliale, settata a corti e tozzi segmenti.

Non altro rileviamo per la identificazione del fungo.

Si aggiunga solo che i miceti nei tessuti non colorati è del tutto jalino, privo cioè di pigmenti bruni o neri propri.

Si direbbe pertanto trattarsi di un ifomicete mucedineo (in quanto ad elementi non colorati) che nei tessuti reattivi dell'ospite assume soltanto la forma miceliale e clamidosporea senza fruttificazioni che possano oggi permettere l'identificazione specifica del micete.

Non è possibile ulteriormente precisare l'etiologia della malattia: non è improbabile che si tratti di un *Aspergillus*, tenendo in calcolo la relativa maggiore percentuale di questo ifomicete tra quelli che possono dare pneumomicosi.

Si è prospettata anche l'ipotesi che potrebbe trattarsi anche di un *Mucor* (o di forme affini); tuttavia l'interpretazione data di una parte degli elementi parassitari come elementi miceliati di un apparato ramificato, settato, starebbe contro tale ipotesi; i *Mucor* hanno infatti un micelio non settato; potrebbe tuttavia restare a questo proposito qualche dubbio. Si ricordi che nella regione dove ha vissuto il nostro paziente sono state osservate, nel 1924, alcune forme di mucormicosi polmonare da A. PERIN, dalle quali è stata isolata la *Lichtheimia italica*. I casi non hanno assunto il quadro anatomo-clinico del nostro, limitati come erano a modeste manifestazioni di addensamento parailare; i casi per la loro sopravvivenza, non sono stati studiati anatomo-patologicamente ed istologicamente, per cui non si hanno elementi di confronto. Uno studio sperimentale con la *Lichtheimia italica* condotto da P. REDAELLI nel 1925 ha dimostrata la patogenicità della specie ma non ha dato reperti parassitari paragonabili con l'attuale.

In ultima analisi, dopo aver detto che con molta probabilità il fungo è un ,,*ifomicete mucedineo*'', non ci resta che indicare la forma in modo generico come una ,,pneumomicosi seguita da generalizzazione granulomatosa''.

Considerazioni sulla modalità di infezione e di diffusione della micosi.

Integrando i dati anamnestici, clinici e radiografici con quelli anatomo-istologici appare chiaro che l'ifo-micete è penetrato, per la via aerea nel lobo superiore polmonare destro, dove ha verosimilmente per un breve periodo svolto un'azione irritativa con reazione infiammatoria essudativa, catarrale banale, sottoforma di bronchite e broncopolmonite, dimostrando un comportamente analogo a quello di alcune note micosi (aspergillosi, moniliasi, ecc.). Ma ben presto, — per ragioni che ci sfuggono — la reazione dell'organismo di fronte al micete ha preso un netto orientamento produttivo, granulomatoso, cercando — ma invano — di bloccare, di digerire il parassita estraneo mediante l'attività enzimatica ed assimilatrice delle cellule infiammatorie del granuloma. Si è venuto così a stabilire una specie di equilibrio fra la capacità di riproduzione ed invasione del parassita e la difesa istiale, che tuttavia ha permesso al primo una lenta invasione in tutta l'economia dell'organismo aggredito.

La storia clinica ed il riscontro anatomico ci permettono di ricostruire le tappe di questa invasione con una discreta sicurezza.

Dalla sede primitiva nel lobo polmonare superiore destro in zona parailare, il processo si è diffuso in senso centrifugo nel parenchima polmonare per continuità. La completa obliterazione del bronco superiore con la conseguente atelettasia del lobo può forse avere favorito tale progressione. Nel contempo devono essere state raggiunte o per continuità, o attraverso lo scarico delle vie linfatiche ed il conseguente interessamento dei linfonodi ilari, le strutture cellulari più lasse del connettivo mediastinico e della sierosa pleurica.

Questo substrato istiale si è dimostrato particolarmente favorevole alla progressione del parassita e alla reazione infiammatoria granulomatosa, così che in tutta la pleura circostante il lobo superiore e nel tessuto interstiziale del mediastino, la lesione ha assunto uno sviluppo imponente. Le lesioni cardiache — primitivamente epicardiche — possono essersi iniziate sia per innesto intracavitario sieroso dai mantelli perivascolari accompagnanti i vasi sino nel loro tratto intrapericardico, sia per diffusione linfatica sottosierosa, sia infine — come per le lesioni discusse avanti — per via ematogena. E' certo infatti che, ad un certo punto, il parassita è passato nel circolo ematico (vene polmonari-cuore sinistro-grande circolo) propagandosi in varii organi. Sono stati così raggiunti i reni ed il fegato e l'encefalo.

Nel fegato e nel rene sinistro, aggredito in più punti, il processo infiammatorio ha assunto un carattere espansivo ma limitato, acquistando una fisionomia simile a metastasi tumorali. Nel rene destro, per ragioni a noi non chiare, il tessuto patologico ha assunto invece il descritto carattere infiltrante e dissociante simil-leucemico con parziale conservazione delle strutture anatomo-istologiche dell'organo. Qui si è successivamente verificato un comportamento analogo a quello pleuro-mediastinico; raggiunto cioè il cellulare lasso della capsula adiposa questa è stata totalmente e massivamente invasa del granuloma, mentre nel tessuto periaortico si è pure avuta infiltrazione più intensa nel cellulare lasso che nei linfonodi.

Il rene destro è poi stato il punto di partenza di una nuova tappa, questa volta per una nuova via e cioè quella intracanicolare. Le cellule fungine, trasportate dalla corrente urinaria si sono innestate nella mucosa del terzo inferiore dell'uretere, dando luogo ad una cospicua ed estesa localizzazione.

Infine ematogena certamente deve ritenersi la localizzazione encefalica che portò a morte il paziente, lesione non potuta constatare autopticamente per la accennata limitazione posta dai parenti del defunto alla esecuzione del controllo necroscopico, ma clinicamente certa.

In conclusione si può affermare che la via di penetrazione fu aerogena e le vie di diffusione nell'organismo furono varie e complesse; per continuità espansiva, per continuità elettiva in determinati substrati (cellulari lassi, sierose), per via linfatica, per innesto intracavitario nelle sierose, per via ematica e per via canalicolare.

In ognuna delle sedi raggiunte dal micete, la ulteriore propagazione al livello istologico, appare avvenire per apposizione di nuovi microgranulomi a quelli preesistenti.

Si dovrebbe poi pensare che il micete intorno a sè, in un tempo relativamente rapido, susciti quella reazione infiammatoria che appare con la fisionomia morfologica descritta e che, in questa situazione, il microgranuloma formato persista indefinitivamente nel tempo senza evolvere nè verso un tessuto cicatriziale definitivo nè verso la necrosi (rari sono i punti necrotici osservati). Solo così si può spiegare l'uniformità del quadro sia nelle lesioni clinicamente più recenti, sia in quelle che risalgono ad oltre un decennio prima della morte.

A questo interessante comportamento biologico che dimostra l'esistenza di un peculiare equilibrio fra organismo ospite e parassita, fa riscontro il non meno particolare comportamento clinico del quadro; il paziente infatti, nonostante la grande estensione delle lesioni patologiche interessanti parecchi visceri, non ha mai dimostrato disturbi generali di una certa entità (febbre, astenia, dimagramento, ecc.).

Anche la sintomatologia degli organi colpiti è stata relativamente modesta nei polmoni e quasi assente per il rene; anche l'esame delle urine, più volte praticato, non ha messo in rilievo alterazioni notevoli, come ci si sarebbe potuti aspettare data la gravità delle lesioni anatomiche.

Fino a che non si è instaurata la localizzazione encefalica che ha portato a morte il soggetto con il quadro clinico di un tumore espansivo intracranico, egli ha goduto un benessere che gli ha permesso il lavoro, ed è presumibile che se la localizzazione encefalica non si fosse verificata, tale situazione si sarebbe prolungata ancora per anni.

Riassunto

Gli AA. descrivono un caso di pneumomicosi cronica, con generalizzazione pluriviscerale, assolutamente eccezionale.

Per causa dell'insolito quadro clinico ed anatomico, che non ha riscontro nella letteratura, la natura della malattia non potè essere diagnosticata che con l'esame istologico delle lesioni.

Résumé

Les AA. décrivent un cas de pneumomycose chronique, avec généralisation pluriviscérale tout à fait exceptionnelle.

A cause de ce tableau clinique et anatomique si extraordinaire, qui n'à pas encore été signalé dans la litérature, il n'à été possible d'en établir le diagnostic qu'au moyen de l'examen histologique des lésions.

Zusammenfassung

Die Verfasser beschreiben einen Fall von chronischer Pneumomykose mit absolut ausserordentlicher pluriviszeraler Generalisation.

Infolge des ungewöhnlichen, klinischen und anatomischen Bildes, das bis heute in der Literatur nicht erwähnt worden ist, hat die Krankheit nur durch die histologische Beobachtung der Veränderungen diagnostiziert werden können.

Summary

The autors report on a case of chronic Pneumonomycosis, with an absolutely exceptional pluriviscerale generalisation.

Due to the unusual clinical and anatomical picture, hitherto not mentioned in the literature, the nature of the illness could be established only by the histological evidence of the alterations.

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THE COMPARISON OF FOUR STRAINS OF COCCIDIOIDES IMMITIS WITH DIVERSE HISTORIES[1]

by

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(1.9.1955)

Only 40 per cent of human beings infected by the fungus *Coccidioides immitis* develop clinical symptoms. About one per cent of adult white males and ten per cent of negro males infected with manifest symptoms progress to the disseminated, progressive extrapulmonary coccidioidal granuloma which is often fatal (1). The cause of dissemination is unknown, but among the theories considered is variation in the pathogenicity of the strains. Recently a strain was submitted to this laboratory which was isolated from a rapidly disseminating and fatal case of coccidioidomycosis. The morphology in culture and the virulence of this strain in laboratory hosts has been compared with other strains of widely differing histories.

Methods

Source of strains — Strain Soil 3a was cultured fifteen years ago directly from the earth where a group of University biology students were infected while digging a rattlesnake out of a ground squirrel hole (2). None of their infections disseminated. While the identity of the strain was established by animal inoculation of parallel cultures, the lineal line of the experimental culture had never been

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The opinions contained in this report are not to be construed as reflecting the views of the Navy Department or the Naval Service at large (Article 1252, U.S. Navy Regulations, 1948).

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passed through laboratory animals. There had been a minimum of thirty transfers on media during this time.

Strain Euphrat was recovered three years ago from a localized cutaneous lesion of a patient of Dr. EUGENE FARBER. The patient's serum fixed complement only in a 1 : 2 dilution, and with this favorable prognostic evidence (3) the lesion was excised. There has been no recurrence.

Strain 46 was isolated twenty-one years ago from a patient who died of a disseminated coccidioidal infection.

The Johnson strain was supplied us in 1954 by Dr. MARSHALL FIESE from a patient of the Fresno Veterans' Administration Hospital with a fulminating coccidioidal infection. His serum fixed complement in a titer of 1 : 256 and he died in two months. There had been only two transfers on media since isolation from the patient.

Preparation of inocula — Material was harvested from petri dish cultures (1 per cent glucose, 0.5 per cent yeast extract and 2 per cent agar) which had been incubated at 35° C until dry.

Assay of virulence — Twenty-five white male mice, ten weeks old, were inoculated intraperitoneally with one milliliter of a suspension containing 100 viable particles as determined by triplicate pour plates at the time of inoculation. The relative virulence of the strains was compared by the percentage of animals dead thirty days after inoculation. The relation of time to mortality as an expression of virulence has been described by FRIEDMAN, SMITH & GORDON (4).

In the case of strain 46, higher dosages, up to one million particles, were also given. Strain 46 was assayed also following an attempt to increase the virulence by twelve intraperitoneal passages through mice. Passage was from mouse to mouse without intervening culture on artificial media and extended over a period of six months.

Results

At the 100 particle dose level, strains Soil 3a, Johnson, and Euphrat were equally lethal for mice (76, 64 and 72 per cent dead, respectively, thirty days after inoculation whereas a strain 46 appeared avirulent (table 1). However, at autopsy ninety days after inoculation, 68 per cent of the animals which received strain 46 showed gross lesions (typified by figure 1). All of the mice which were inoculated with the other three strains also were infected.

Not until the dosage of strain 46 was increased to a million particles did the rate of dying approximate that of the animals inoculated with 100 particles of the other strains. Lesions in the animals which died following the high dose of strain 46 were similar to those seen in animals with the lower dosages of the other three strains (figures 2 and 3).

Passage of strain 46 through mice did not alter the virulence of that strain for mice.

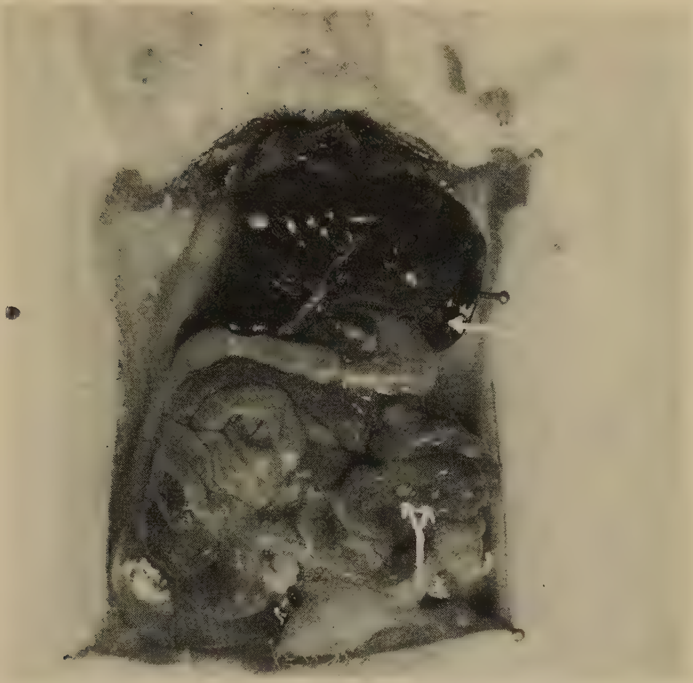

Figure 1. A mouse which was inoculated with 100,000 particles of strain 46 and sacrificed after 90 days. The liver is pinned back to expose the characteristic fibrotic lesions.

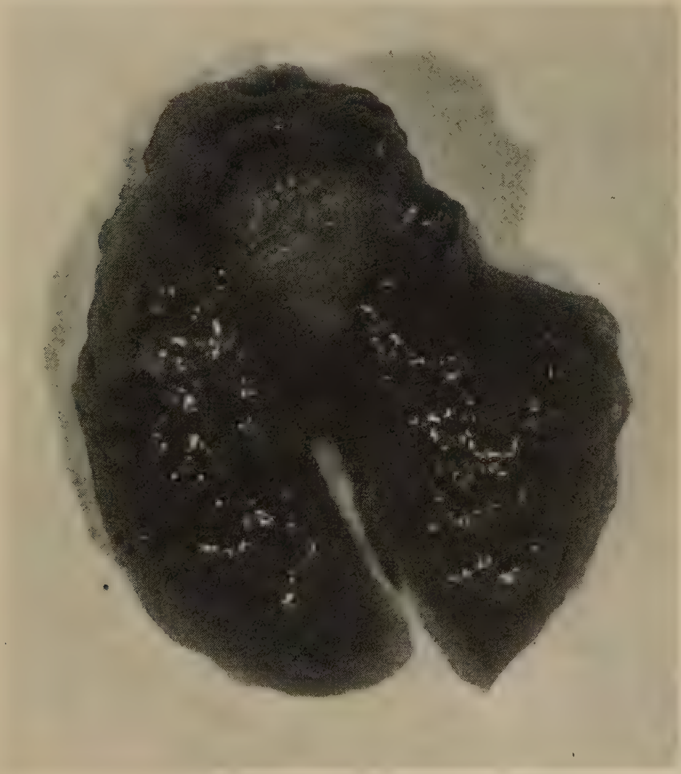

Figure 2. The lungs of a mouse which died 20 days after inoculation with one million

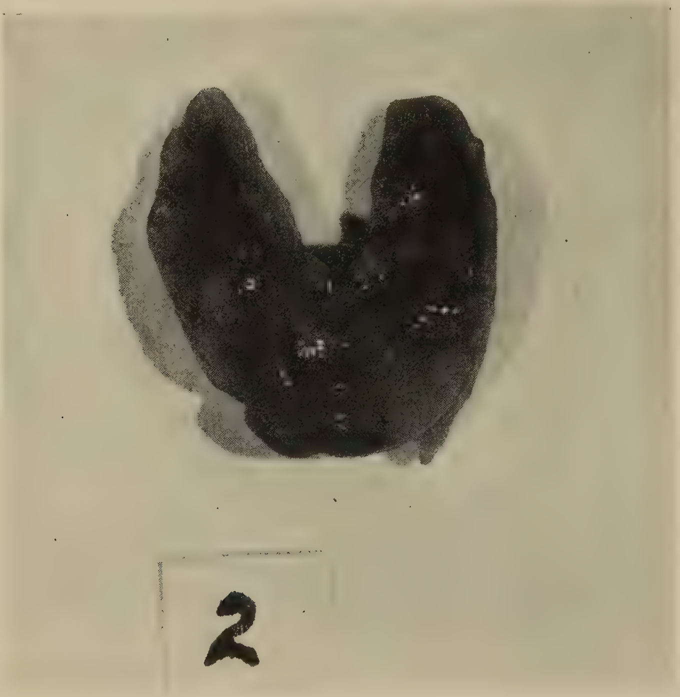

Figure 3. The lungs of a mouse which died 33 days after inoculation with 10 particles of strain Soil 3a.

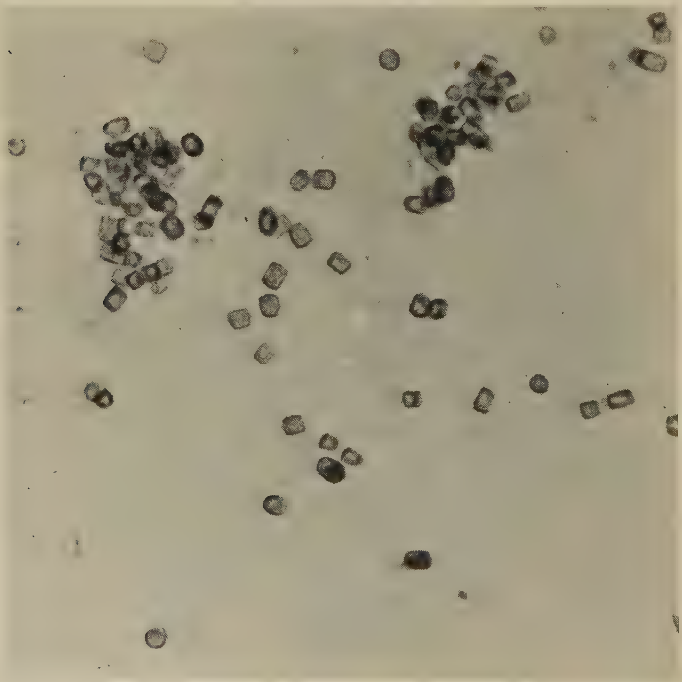

Figure 4. Microscopic preparation of a culture of strain Euphrat. Hyphae have converted entirely to classical arthrospores. (× 440).

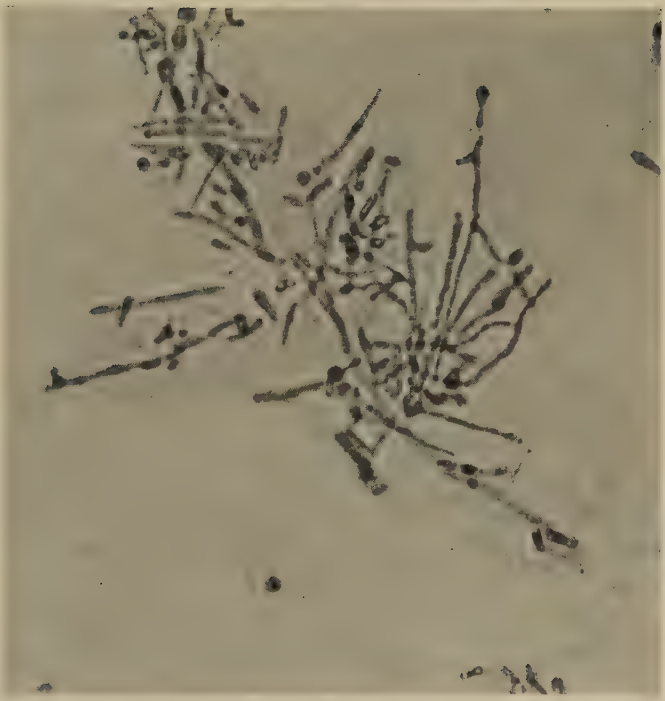

Figure 5. Microscopic preparation of a culture of strain 46, the same age as the cells of figure four. Hyphae did not convert to classical arthrospores even after further incubation. (× 440)

TABLE I History and virulence of cultures

Strain	Date of isolation	Clinical history	Virulence for mice, percentage of animals dead 30 days after inoculation with 100 viable particles
Johnson	Jan '54	Coccidioidal granuloma, fatal within 6 months	64
46	1933	Coccidioidal granuloma, fatal	0
Soil 3a	1939	None, isolated from soil	72
Euphrat	1951	Coccidioidal granuloma, dissemination limited to cutaneous area, patient recovered	76

The sporulating capacities in culture differed markedly. Soil 3a, Euphrat (figure 4) and Johnson produced spores of classical morphology, although the quantity produced by the Johnson strain was sparse. Strain 46 spores were not only low in quantity but aberrant in morphology (figure 5).

Summary

By the criterion of lethality in mice, a strain of *Coccidioides immitis* recently isolated from a most devastating form of human coccidioidomycosis was of no greater virulence than another isolated from a much less severe case or even one cultured from soil fifteen years ago and which presumably had caused seven self-limited, benign cases of primary coccidioidomycosis. There was no enhancement of virulence to mice by twelve passages of a relatively avirulent strain which had been recovered twenty-one years ago from a fatal human case.

Conclusions

There appears to be no correlation between severity of human coccidioidomycosis and virulence of the etiologic strain for mice. Possibly dosage of inoculum and certainly host factors play determining roles in the human infection spectrum.

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DEUX CAS DE RHINOSPORIDIOSE NASALE CHEZ UNE OIE ET UN CANARD SAUVAGES A ASTRIDA (RUANDA-URUNDI)

par

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(1.9.55)

Au cours de recherches sur la „trichobilharziose" nasale des oiseaux à Astrida, l'un de nous (Fain) découvrit dans les fosses nasales d'une oie d'Egypte (*Alopochen aegyptiacus* Linn.) et d'un canard siffleur africain (*Dendrocygna viduata* Linn.) des tumeurs polypeuses dont la surface était parsemée de petits points blanchâtres. L'étude histopathologique de ces tumeurs montra qu'il s'agissait de granulomes rhinosporidiens. C'est chez le canard siffleur que les lésions étaient les plus intenses. La muqueuse nasale de cet oiseau était couverte de nombreux polypes, sessiles ou pédiculés, de dimension variable, présentant tous un piqueté blanchâtre visible à l'oeil nu. L'examen microscopique, entre lame et lamelle, de ces petites granulations montra que celles-ci étaient en réalité des petits kystes renfermant un grand nombre de petites formations arrondies ou ovalaires, de la dimension d'un globule rouge environ. Les dimensions, la structure et le contenu de ces kystes nous firent penser à la rhinosporidiose. Ce diagnostic fut confirmé par l'examen histologique des polypes qui montra l'image caractéristique de cette parasitose. Nous donnons ci-dessous une brève description de ces lésions:

> „Les polypes sont formés essentiellement d'un tissu conjonctif jeune fortement infiltré de cellules inflammatoires parmi lesquelles les lymphocytes sont nettement prédominants mais ou on rencontre également des plasmocytes, quelques polynucléaires neutrophiles et d'assez nombreux éosinophiles. Au sein de ce tissu granulomateux on découvre de nombreux sporanges plus ou moins volumineux, pouvant atteindre au maximum un diamètre de 0,325 mm (fig. 1). Ces grands sporanges sont bourrés de spores complètement développées globuleuses ou subglobuleuses et d'aspect muriforme. Le diamètre de ces spores varie entre 0,007 et 0,009 mm, exceptionnellement il peut atteindre 0,01 à 0,012 mm. L'examen à un fort grossissement montre que ces spores sont formées de petits granules plus ou moins arrondis, colorables à l'éosine, serrées les uns contre les autres, et d'un noyau un peu plus volumineux, de 0,002 à 0,003 mm de diamètre,

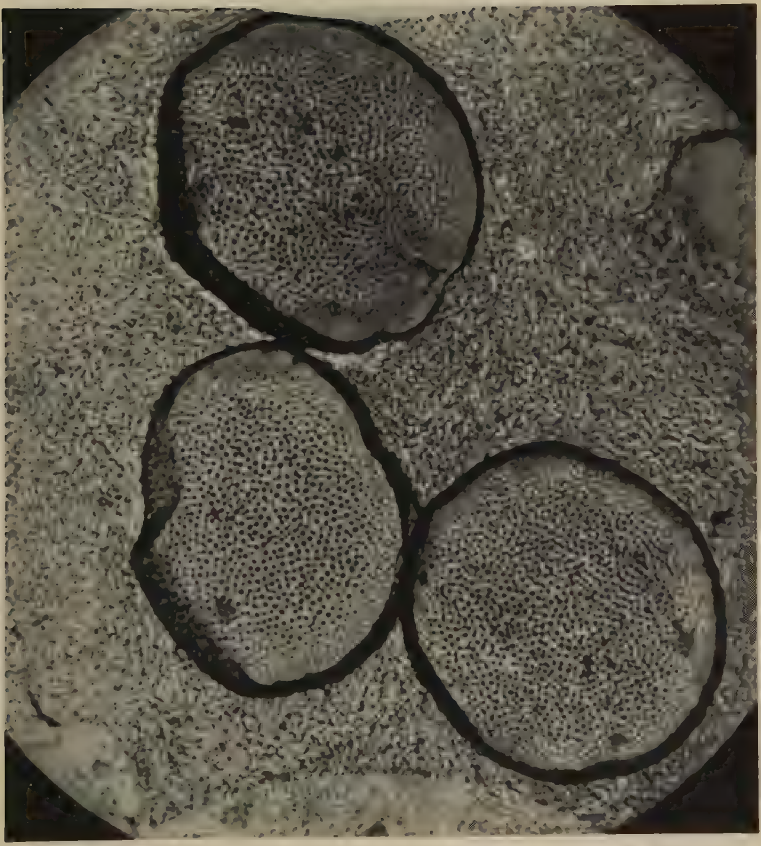

Fig. 1. Grands sporanges bourrés de spores complètement développées. (Microphoto)

très pâle et visible seulement grâce à sa fine membrane nucléaire et à son gros nucléole tous deux colorés par l'hématoxyline (fig. 2). Ce noyau est souvent masqué, plus ou moins complètement, par les granules ou sphérules surtout dans les préparations un peu trop colorées à l'éosine. Les spores les plus volumineuses sont tassées au centre du sporange, à mesure qu'on s'éloigne du centre les spores deviennent plus petites et elles perdent progressivement leur caractère éosinophilique et tout à fait à la périphérie du sporange elles se résolvent en une fine poussière colorée par l'hématoxyline. Une membrane anhiste plus ou moins épaisse entoure les sporanges. Certains sporanges communiquent directement avec l'extérieur par un pore donnant issue aux spores (fig. 3). En dehors des sporanges matures dont nous venons de parler les polypes renferment encore des sporanges plus petits dont les spores sont encore à un stade très jeune, ainsi que des sporanges en voie de résorption dont les parois sont collabées et dont le contenu a été partiellement ou complètement vidé à l'extérieur. Ces débris de sporanges sont tous envahis par de nombreuses et volumineuses cellules géantes (fig. 4)."

Il convient encore de signaler que dans ces polypes déja envahis par des rhinosporidies à tous les stades de leur développement nous avons également pu mettre en évidence quelques oeufs de schistosomes appartenant probablement à l'une des espèces de *Trichobilharzia* que nous avons décrites récemment. Quelques rares oeufs étaient, comme les débris des sporanges, envahis par des cellules géantes. Il est donc très probable que la présence de ces oeufs dans les polypes a pu contribuer à augmenter les lésions déclanchées par la rhinosporidiose, nous pensons toutefois que leur influence a été négligeable ou nulle dans la production du polype lui-même, en effet l'étude de la trichobilharziose nasale a montré à l'un de nous (FAIN) que cette affection ne s'accompagne jamais de formations polypeuses sur la muqueuse nasale même dans les cas d'infestation intense. On peut donc en conclure que les polypes sont produits exclusivement par la rhinosporidiose et que les oeufs de schistosomes ne jouent aucun rôle dans leur formation.

Chez l'oie d'Egypte les lésions sont essentiellement les mêmes que celles que nous venons de décrire mais les sporanges sont apparemment tous au même stade et beaucoup plus petits que dans le cas précédent et aucun ne contient de spores mûres. De plus les lésions de fibrose sont un peu plus accusées et le tissu de granulation est en conséquence plus réduit. Ici aussi nous avons trouvé des oeufs de schistosomes dans les polypes mais en nombre très restreint."

La coexistence de la rhinosporidiose et de la trichobilharziose nasales ne doit pas nous étonner, l'un de nous a montré tout récemment que cette dernière affection est quasi généralisée chez la

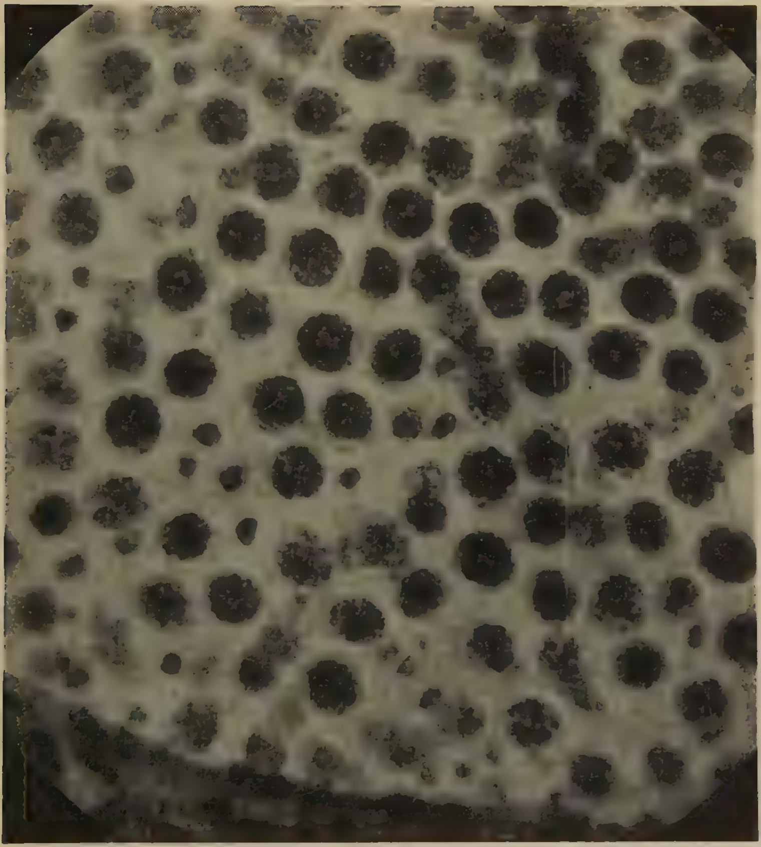

Fig. 2. Spores vues à un très fort grossissement. On distingue nettement les sphérules hyalines et le noyau plus pâle, généralement unique, parfois double, contenant un gros nucléole très coloré. (Microphoto).

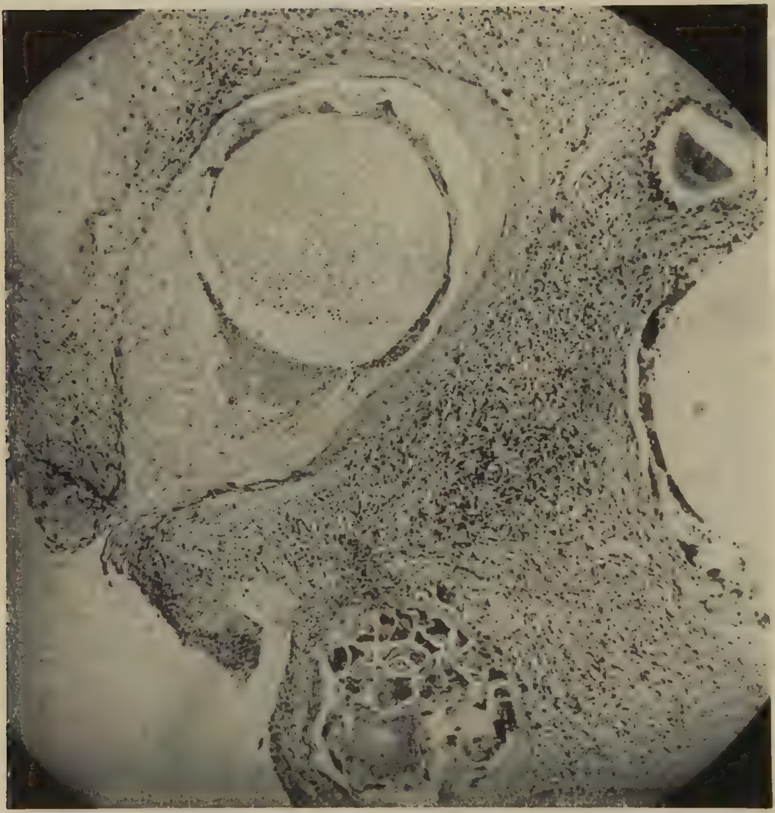

Fig. 3. Dans le haut du cliché: grand sporange en communication avec l'extérieur. Dans le bas du cliché: sporange vide envahi par les cellules géantes. (Microphoto).

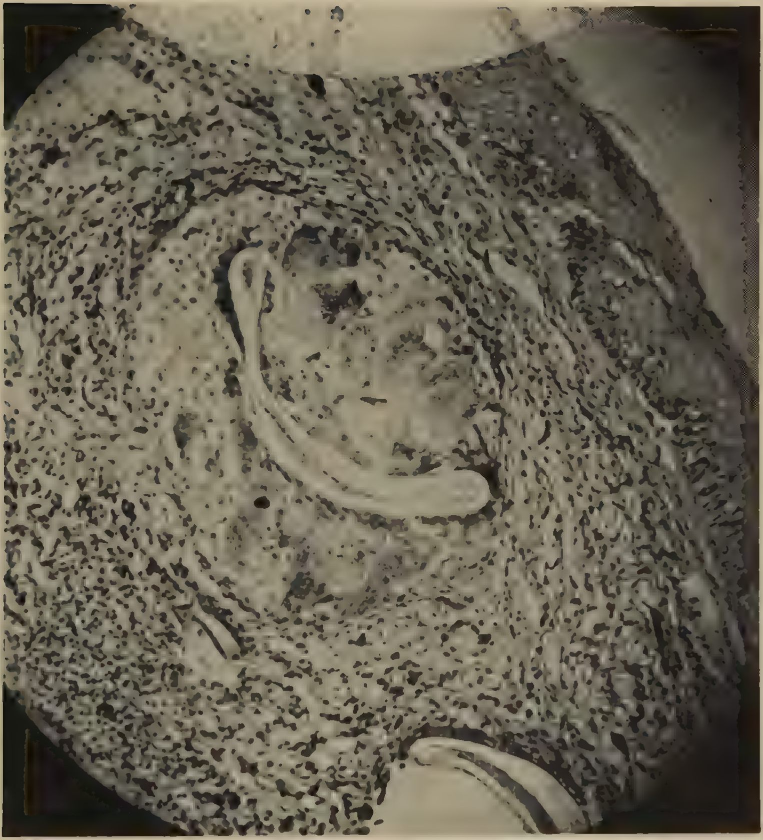

Fig. 4. Parois de sporange collabées en entourées de volumineuses cellules géantes. (Microphoto).

plupart des anatidés au Ruanda-Urundi. Il paraît aussi intéressant de noter que les deux oiseaux parasités sont très communs dans toute l'Afrique Centrale, de plus leur aire de distribution est très vaste et ils peuvent présenter des migrations au cours desquelles ils effectuent de grands déplacements. L'oie d'Egypte se retrouve dans tout le continent africain et on peut même la rencontrer dans les parties méridionales de l'Europe. Le canard siffleur africain est également très répandu en Afrique mais il est surtout abondant en Afrique du sud. Si nous insistons sur la distribution des deux hôtes de la rhinosporidiose c'est parce qu'elle peut avoir une certaine importance dans l'épidémiologie de cette parasitose. Le parasite que nous avons découvert chez ces canards est en effet identique à celui qu'on rencontre chez l'homme et le animaux domestique et qui est connu sous le nom de *Rhinosporidium seeberi* (WERNICKE, 1900). La découverte chez des oiseaux aquatiques et migrateurs, de ce parasite, qui est essentiellement un parasite de l'homme, est importante à un double point de vue. En effet non seulement elle apporte un argument de poids à la théorie de la transmission hydrique de cette parasitose mais elle laisse entrevoir en outre la possibilité d'une transmission du parasite d'un pays à l'autre. Cette transmission permettrait d'expliquer d'une certaine façon la répartition apparemment paradoxale de cette affection qui malgré sa grande rareté existe cependant à l'état sporadique dans un grand nombre de pays.

Ajoutons encore que les deux cas de rhinosporidiose que nous venons de décrire ont été observés par nous pendant le mois d'octobre 1954, c'est à dire à la fin de la saison sèche au Ruanda-Urundi. Aucun autre cas ne fut découvert dans les mois qui suivirent (novembre à avril) malgré le grand nombre d'oiseaux aquatiques examinés.

Rappelons que la rhinosporidiose est avant tout une affection de l'homme. Les lésions les plus fréquentes sont celles des fosses nasales mais on peut également rencontrer le parasite dans l'oreille, dans la conjonctive ou encore sur le pénis. Elle est surtout répandue aux Indes et en Ceylan, et dans ces pays elle est pour ainsi dire endémique, mais on la rencontre aussi sporadiquement dans différentes parties du monde. En Afrique elle a été signalée dans les régions du sud (Afrique du sud), en Uganda et au Congo Belge. Dans les 3 cas congolais le parasite était localisé dans la conjonctive.

Le parasite a également été rencontré chez des animaux domestiques cheval, mulet et bovidés. Un seul cas a été signalé chez les oiseaux par GRAHAM SMITH en 1907 (cité par LESBOUYRIES, 1941), le parasite, attribué à *Rhinosporidium seeberi* avait été trouvé dans les muscles surtout les muscles cardiaques chez une perruche (*Psittacus undulatus*).

La position systématique du parasite: *Rhinosporidium seeberi* (WERNICKE, 1900) est encore incertaine. Pendant longtemps il fut rangé parmi les protozoaires, d'abord dans le groupe des coccidies, puis dans celui des haplosporidies. C'est ASHWORTH qui en 1923 démontra que ce parasite n'était pas un protozoaire mais un champignon de l'ordre des phycomycètes.

Le genre *Rhinosporidium*, si on excepte le genotype *Rh. seeberi*, ne comprend pas moins de 4 espèces, décrites chez l'homme ou les animaux. Beaucoup d'auteurs considèrent toutes ces espèces comme des synonymes de l'espèce type. On a même assimilé à *Rh. seeberi*, un parasite découvert chez une grenouille arboricole: *Hyla rubra* et qui a été décrit sous le nom de *Dermosporidium hylarum* Carini, 1940.

Le parasite que nous avons découvert dans les fosses nasales des deux anatidés est inséparable au point de vue morphologique du parasite humain *Rhinosporidium seeberi*. Cette similitude morphologique n'est évidemment pas une preuve absolue que les 2 parasites sont identiques, car comme le dit très justement VANBREUSEGHEM (in LANGERON et VANBREUSEGHEM, 1952) à propos de la pluralité des espèces de *Rhinosporidium*, les ressemblances constatées peuvent être liées uniquement à des phénomènes de convergence. Quoiqu'il en soit et malgré la grande différence qui, du point de vue zoologique, sépare les mammifères des oiseaux, nous préferons nous abstenir de créer une nouvelle espèce pour ce parasite des anatidés, et nous le considérerons comme identique à *Rhinosporidium seeberi*.

Conclusions

Les auteurs relatent la découverte à Astrida de deux cas de rhinosporidiose nasale chez des anatidés très répandus en Afrique centrale: l'oie d' Egypte et le petit canard siffleur africain.

Les lésions observées consistent en polypes des fosses nasales. Histologiquement ces polypes correspondent à des granulomes et leur structure est dans les grandes lignes identique à celle qu'on observe dans les lésions de rhinosporidiose nasale chez l'homme.

Le parasite observé dans les lésions est morphologiquement identique à *Rhinosporidium seeberi*, l'agent causal de la rhinosporidose chez l'homme.

Les auteurs envisagent les conséquences de cette découverte au point de vue de l'épidémiologie de la rhinosporidiose humaine.

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THE EFFECT OF HORMONES ON EXPERIMENTAL MONILIASIS IN MICE

I. Sex Hormones, Cortisone, and Somatotrophic Hormone[1, 2]

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(15.12.1954)

During the course of studies on the effect of cortisone and somatotrophic hormone (STH) on experimental moniliasis in mice (SCHERR, 1953b, 1953c, 1953d) it was frequently demonstrated that major differences in results under a certain regimen of treatment could be attributed to differences in the sex of the animals. For example, it was shown that normal male mice are more susceptible to the toxic properties of cortisone than female animals (SCHERR, 1952), and that an amount of STH which would reduce the deleterious effect of cortisone on infected female mice was not effective for males (SCHERR, 1953c). HASE and SCHINDEL (1949) had also found differences in the effect of ACTH on immature male and female rats and offered the explanation that an antagonism exists between ACTH and testosterone but not between ACTH and estrogen.

It was felt possible to alter the response of infected male and female animals to cortisone and/or STH by the concomitant use of sex hormones. This report presents the work done with testosterone and estradiol.

Materials and Methods

The materials and methods used here were essentially those previously reported (SCHERR, 1953a, 1953e). *Candida albicans*, hitherto designated strain 171D, is now ATCC strain # 11651.

Treatment was instituted 24 hours after infection with *C. albicans*. Cortisone (Schering) was inoculated intramuscularly, 0.3 mg (.05 ml) every 48 hours for the first two weeks and a doubled dose in the same volume thereafter; 015 mg (.05 ml) STH (Frank W. Horner, Ltd.) was inoculated intramuscularly every day for the first two weeks and a doubled dose in the same volume thereafter. To compensate for tolerance to the testosterone and estradiol ensuing from extended inoculations, and also to avoid the need for

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[2] A preliminary report of this work has been presented (SCHERR, 1954a).

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castration of the animals, testosterone (Oreton-F^R, Schering) or estradiol (Progynon^R, Schering) was inoculated intramuscularly in a volume of .05 ml every 48 hours according to the following schedule:

	Testosterone	Estradiol
1 st dose	.025 mg	.001 mg
2nd	.05	.002
3rd	.10	.004
4th	.20	.008
5th	.20	.008
6th	.20	.008
7th	.20	.008
8th and to the end of experiment	.40	.016

Where mixtures of the various agents were used, they were prepared so that they could be injected simultaneously in a volume of .05 ml. The vehicle for all these agents was that in which the cortisone is suspended. Mice treated solely with the aqueous vehicle received 0.1 ml intramuscularly every 48 hours.

The method of assessing the severity of infection and the mortality rate by "dissemination values" has been previously described by SCHERR (1953b, 1953c, 1953e).

Results

Tabels 1 and 2 summarize the data for infected and uninfected mice treated with cortisone, somatotrophic hormone, and testosterone, alone and in combination.

Probably one of the most significant features of these data is that testosterone enhanced the deleterious effect of cortisone on infected male and female mice and that this synergistic deleterious effect increased with an increased dosage of testosterone. Thus, the mean dissemination values for groups of infected male mice untreated, treated with cortisone, a mixture of testosterone and cortisone, and a doubled dose of testosterone in this cortisone-testosterone mixture were 6.3, 12.7, 15.9, and 18.8, respectively. When administered alone, the amount of testosterone used did not alter the severity of the infection in male mice (compare groups 4–122A, 4–123A, and 4–159E in table 1). The same dosage schedule of cortisone and testosterone used for male animals also enhanced the severity of the infection for females, but to a lesser extent. Thus, the mean dissemination values for the groups of infected female mice untreated, treated with cortisone, with testosterone and cortisone, and with a doubled dosage of testosterone but unaltered dose of cortisone, were 3.4, 11.1, 11.5, and 15.7, respectively.

This deleterious effect of cortisone and testosterone for infected male and female mice is better visualized by the graph of figure 1.

It can be seen that the increase in the severity of the infection between the untreated groups and those receiving the doubled dose of testosterone in the cortisone-testosterone mixture is essentially the same for male and female mice (compare columns A and D for both sexes). However, there appeared to be a critical threshhold in the female mice for the amount of testosterone, since there is no difference between female mice treated with cortisone and those treated with the lower dosage of testosterone and cortisone (compare columns B and C for the female mice).

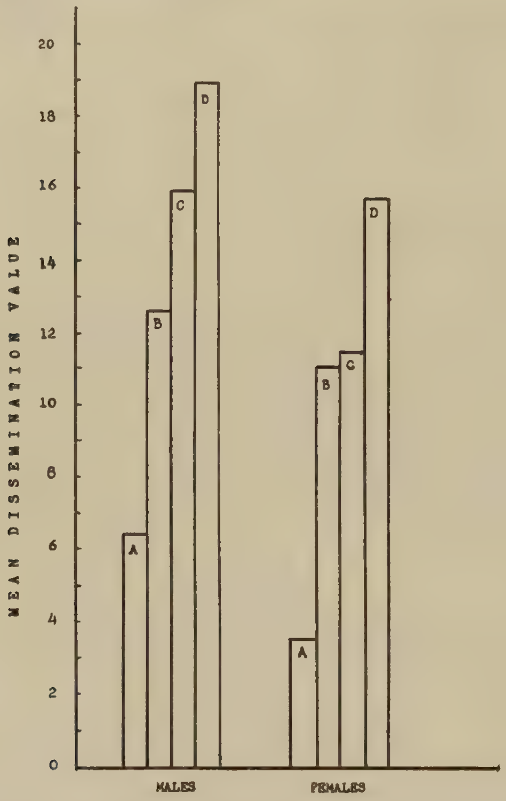

Figure 1. The effect of cortisone and testosterone on male and female mice infected with moniliasis and treated with: A-untreated, B-cortisone, C-cortisone and testosterone, D-cortisone and testosterone (2 ×). These groups of animals are same as presented in table 1; see text for dose schedules.

Since the regimen of STH used here for infected male mice enhanced the severity of the infection, we can only conclude that testosterone suppressed this effect of STH, since the mean dissemination values for infected males treated with STH and those treated with a mixture of STH and testosterone were 11.1 and 6.8, respectively. Note that this reduction in dissemination which we attribute to testosterone went no lower than the value for the infected, untreated controls (4–122A in table 1).

TABLE 1. *The Effect of Cortisone, Somatotrophic Hormone* (STH), *and Testosterone on Experimental Moniliasis in Mice* (*Duration of Experiment*— 29 *days*)

Infected mice treated with[1]	No. mice	Sex	Mean dissemination value	Mortality (%)
Untreated (4–122A)	20	M	6.3	30.0
Testosterone (4–123A)	20	M	7.9	30.0
Testosterone (2×), (4–159E)	19	M	6.5	25.0
Testosterone and cortisone (4–122S)	20	M	15.9	85.0
Testosterone (2×) and corti- (4–159C)	20	M	18.8	95.0
Cortisone (4–122E)	20	M	12.7	80.0
Testosterone and STH (4–122U)	20	M	6.8	40.0
STH (4–122C)	20	M	11.1	55.0
Cortisone, testosterone, and STH (4–122W)	20	M	10.6	65.0
Cortisone and STH (4–122G)	20	M	14.5	90.0
Untreated (4–122B)	20	F	3.4	10.0
Testosterone (4–123B)	20	F	16.2	85.0
Testosterone (2×), (4–159F)	(no data available)			
Testosterone and cortisone (4–122T)	20	F	11.5	70.0
Testosterone (2×) and cortisone (4–159D)	20	F	15.7	85.0
Cortisone (4–122F)	20	F	11.1	65.0
Testosterone and STH (4–122V)	20	F	7.9	40.0
STH (4–122D)	20	F	7.0	40.0
Cortisone, STH and testosterone (4–122X)	20	F	10.3	55.0
Cortisone and STH (4–122H)	20	F	10.8	70.0

[1] For details of treatment schedules see text. In order to facilitate the presentation and discussion of these and data which follow, each group of animals has been assigned a code number.

STH was able to antagonize partially the effect of cortisone in the cortisone-testosterone mixture for male mice (compare groups 4–122W and 4–122S in table 1). If STH had antagonized all the cortisone in this mixture, we might have expected that the mean dissemination value for infected male mice treated with a mixture of cortisone, STH, and testosterone (4–122W) would be the same as that of infected male animals treated solely with testosterone (4–123A); this incomplete antagonism is reflected by the difference in mortality data more than in dissemination values.

In contrast to infected male animals, infected females were markedly affected by treatment with testosterone (compare groups 4–122B and 4–123B in table 1). This deleterious effect of testosterone was completely suppressed by the dosage of STH used here, the groups of infected female mice untreated, treated with testosterone, with testosterone and STH, and with STH alone had mean dissemination values of 3.4, 16.2, 7.9, and 7.0 respectively.

The severity of the infection for the female mice treated with testosterone and cortisone (groups 4–122T and 4–159D in table 1) was never greater than that of the group treated solely with testosterone (4–123B).

TABLE 2. *The Effect of Cortisone, Somatotrophic Hormone* (STH), *and Testosterone on Normal Mice*[1])

Uninfected mice treated with	No. mice	Sex	Mean disseminati-tion value	Mortality (%)
Testosterone (4–159W) . . .	20	M	0.0	0.0
Testosterone and cortisone (4–159Q)	20	M	7.3	40.0
Cortisone (4–123S)	20	M	0.0	0.0
Testosterone and STH (4–159S)	20	M	7.4	30.0
STH (4–158C)	20	M	11.2	50.0
Testosterone, cortisone, and STH (4–159U)	20	M	4.6	35.0
Cortisone and STH (4–158E)	20	M	1.9	10.0
Testosterone (4–159X). . . .	20	F	0.0	0.0
Testosterone and cortisone (4–159R).	20	F	0.3	5.0
Cortisone (4–123T)	20	F	0.0	0.0
Testosterone and STH (4–159T).	20	F	5.6	25.0
STH (4–158D)	20	F	2.3	15.0
Testosterone, cortisone, and STH (4–159V)	20	F	0.5	5.0
Cortisone and STH (4–158F)	20	F	4.1	20.0

[1]) These animals were treated simultaneously with and similarly to those described in table 1.

This deleterious synergism between cortisone and testosterone is also vividly apparent from the data for male mice in table 2; a mixture of cortisone and testosterone at dosage levels which had no toxic effect on normal male mice when used alone resulted in a mean dissemination value of 7.3 and 40 % mortality (compare groups 4–159W, 4–123S, and 4–159Q in table 2). This toxic effect was suppressed to a very slight degree by STH (group 4–159U in table 2).

The dosage of testosterone used (table 2) was not toxic to female mice either, but it is noteworthy that whereas in male mice a mixture of testosterone and STH (4–159S in table 2) was slightly less toxic than STH alone (4–158C), the same mixture was more toxic for female mice (4–159T in table 2) than STH alone (4–158D). This toxicity was completely reversed by cortisone; thus, the mean dissemination and mortality data for normal female mice treated with a mixture of testosterone, cortisone, and STH (4–159V) was essentially the same as that for female mice inoculated solely with testosterone (4–159X).

TABLE 3. *The Effect of Cortisone, Somatotrophic Hormone* (STH), *and Estradiol on Experimental Moniliasis in Mice* (*Duration of experiment* — 29 *days*)

Infected mice treated with[1]	No. mice	Sex	Mean dissemination value	Mortality (%)
Estradiol (4–123I)	20	M	15.2	70.0
Estradiol and cortisone (4–123C).	20	M	11.0	70.0
Estradiol and STH (4–123E)	19	M	3.6	10.5
Cortisone, estradiol, and STH (4–123G).	20	M	15.9	75.0
Estradiol (4–123J)	20	F	3.1	10.0
Estradiol and cortisone (4-123D)	20	F	17.0	90.0
Estradiol and STH (4–123F) . .	20	F	20.1	75.0
Cortisone, estradiol, and STH (4–123H).	20	F	19.1	90.0

[1] For details of treatment schedules see text. Control data for infected and normal male and female mice treated with cortisone and/or STH are recorded in tables 1 and 2.

Table 3 records the data for animals infected similarly to and simultaneously with those presented in tables 1 and 2 except that estradiol was substituted for testosterone.

Infected males treated with estradiol (4–123I in table 3) showed a marked increase in the severity of the infection but infected females similarly treated were unaffected. A mixture of cortisone and estradiol was no more damaging to infected male animals than those treated solely with estradiol (compare groups 4–123I and 4–123C in table 3) but for the infected female animals this same mixture was much more deleterious than cortisone alone (compare groups 4–123J and 4–123D in table 3 with group 4–122F in table 1).

STH antagonized the deleterious effect of estradiol for infected male animals, but more than this, the dissemination value and mortality rate for infected males treated with estradiol and STH was significantly lower than that for infected, untreated male mice (compare 4–123E in table 3 to 4–122A in table 1). In infected female mice the mixture of STH and estradiol markedly enhanced the mean dissemination value and mortality rate.

Infected male mice treated with a mixture of cortisone, STH, and estradiol showed a mean dissemination value and mortality rate greater than that found with the mixture of estradiol and STH but equal to that found for infected males treated solely with estradiol (compare groups 4–123I, 4–123E, and 4–123G in table 3). This result indicates that the effect of STH in suppressing the deleterious property of estradiol for infected male animals was completely reversed by cortisone. Reasoning from these data we might have expected that the deleterious effect of STH for infected female mice treated with estradiol (4–123F) would also be antagonized by cortisone, resulting in a mean dissemination value for infected females treated with a mixture of cortisone, STH, and estradiol comparable to that for infected female mice treated solely with the estradiol. That this was not the case (see 4–123H in table 3) again supports our original contention that, under certain conditions, male mice are more susceptible to the effects of cortisone than female animals.

Discussion

The results of this study using estradiol and testosterone in conjunction with cortisone and STH emphasize still further that the differences in the response of the animals to treatment with cortisone and STH may in a large measure be attributed to sex differences.

Androgenic effects of cortisone have been reported as manifest by an increase in acne (Thayer, 1952). Androgens have also been reported to decrease the weight of the adrenals of rats of both sexes, whether these animals were gonadectomized or not (Burrows, 1949). The study of Rennels *et al.* (1953), on the other hand, led these workers to conclude that androgen treatment would cause adrenal hypertrophy; they indicated, however, that their conclusions did not preclude the possibility that other experimental conditions might give diverse results. For example, an increase in adrenal weight in rats fed a high protein diet could be reversed by testosterone propionate but only under rather exacting conditions

(LEATHEM, 1951). The deleterious effect of testosterone only for infected female mice is puzzling (table 1), especially since either cortisone or STH was able to suppress this deleterious effect for infected females to a certain extent. Estrogens also can cause a marked hypertrophy of the adrenal gland (RENNELS *et al.*, 1953). Similar implications are apparent in the studies of ANTOPOL (1950), LEROY (1952), ATERMAN and GREENBERG (1952), JONES (1949), and others.

The study reported here and those of numerous other workers (see reviews by KASS and FINLAND, 1953 and ANTOPOL and QUITTNER, 1952) indicate that there is an interplay of adrenal-cortical and other hormones which tends to maintain a state of resistance or susceptibility to infectious diseases. This activity is essentially non-specific and affects the resistance of the host through the effect on numerous bodily functions e.g., capillary fragility, inflammatory response, antibody formation, salt and fluid balance, and many others. If alteration in the performance of these bodily functions by induced changes in the concentrations of various hormones enhances the susceptibility of the host to infection with certain pathogenic agents, then it should be feasible to suppress active infectious states by exerting an influence on the above-mentioned bodily functions by judicious infections of the proper kind(s) and concentration(s) of certain hormones.

The statement hitherto accepted almost axiomatically that cortisone is deleterious to an infectious state is rapidly being modified in the light of studies showing that cortisone may be efficacious or deleterious in certain infectious states *depending upon certain conditions* (DUFFY and MORGAN, 1951; SCHERR, 1953b, 1953d; KILBOURNE, 1952; ROBINSON *et al.*, 1953).

The hormones used here doubtlessly act on the infectious organisms indirectly through their effect on the physiology of the host. Although *in vitro* studies assessing the effect on pathogenic fungi of various hormones and other steroids have been done, the results have on occasion given conflicting data or have not been borne out by *in vivo* work. Thus, REISS (1949) reported approximately 50 per cent inhibition of the growth of *Microsporon fulvum* by a concentration of .74 millimoles of testosterone per liter of solution, whereas TARBET *et al.* (1953) reported that the growth of this organism was stimulated with either one or one hundred ppm of testosterone in the culture medium.

Since sexuality varies in degree as well as in kind, these studies suggest the possibility of modifying a patient's response to various treatment schedules with cortisone or somatotrophic hormone by the use of sex hormones. It is also our contention that sex and other hormones could serve to modify favorably the effect of cortisone on numerous infectious states. The clinical application of such a program still requires much experimental work and would necessitate a number of key diagnostic tools in order to determine the proper hormones and their dosage requirements under the special set of conditions existing in that patient. These requirements are especially emphasized by the data of SCHERR (1954b and unpublished data) who showed that a particular dose schedule of cortisone, STH, and hesperedin methyl chalcone almost completely obliterated any signs of experimental moniliasis in female mice but not in males, and that this same treatment schedule was ineffective for both sexes if the severity of the infection were altered. Such key diagnostic tools as mentioned above might be determinations of capillary fragility, eosinophile counts, androgen-estrogen ratio, and others pertinent to the disease and the patient.

Summary

By inoculating testosterone or estradiol alone and in combination with cortisone and somatotrophic hormone into male and female mice infected with moniliasis, it was shown that these sex hormones play a major role in modifying the course of the infection in these animals. The use of testosterone indicated that it had a marked synergistic activity with cortisone in enhancing the severity of the infection for both sexes. In addition, a mixture of testosterone and cortisone inoculated into normal mice at dosage levels which displayed no toxic effect when either agent was used alone, displayed a marked toxicity for male animals.

These data also support the contention that the sex of the animal plays a major role in governing the response of normal or infected animals treated with cortisone and/or somatotrophic hormones.

The possible implication of these data clinically is discussed.

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THE EFFECT OF HORMONES ON EXPERIMENTAL MONILIASIS IN MICE

II. Gonadotropins[1])

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(15.12.1954)

In the course of experiments in which Swiss mice were infected with *Candida albicans* strain 171D (now designated ATCC # 11651) it was noted that pregnant mice showed a higher degree of resistance to infection than non-pregnant females or males (Scherr, 1951; Scherr and Weaver, 1951). As a consequence of this an extended series of studies was undertaken to investigate the effect of various hormones, alone and in combination, on experimental moniliasis in mice. The background information for these studies has already been presented and need not be repeated here (Scherr, 1953a, 1953b, 1954). This report describes the work done with gonadotropins.

Materials and Methods

The materials and methods used were essentially those previously reported (Scherr, 1954). Where statistical evaluation of the validity of differences between two groups of experimental animals was necessary, the method of t was used.

Animals were always infected by the intraperitoneal route, with *Candida* cells suspended in physiological saline; when a more severe infection was desired, 5 per cent mucin was substituted for the saline.

The gonadotropic hormones used in this study were:

Anteron R (Schering), which is prepared from pregnant mare's serum.

Pranturon R (Schering), which is obtained from human pregnancy urine.

Gonadogen R (Upjohn), which is prepared from pregnant mare's serum.

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The first two preparations are standardized in International Units (I.U.); Gonadogen is standardized in terms of Cartland-Nelson (C.N.) units, one C.N. unit being equivalent to approximately 20 I.U.

Since doses and duration of experiment may vary with each experiment, these data are given in the text where pertinent.

Results

It seemed appropriate to do first an experiment to determine the effect of pregnant mouse serum on mice infected with moniliasis. Our data are fragmentary because of the difficulty of collecting large quantities of such serum, but it did serve to indicate that male and female mice infected with moniliasis and treated intraperitoneally daily with 0.2 ml of pooled pregnant mouse serum showed a significant reduction in the severity of the infection as compared to untreated animals (table 1).

TABLE 1. *Effect of Normal Pregnant Mouse Serum on Mice Infected with Moniliasis*
(*Duration of experiment* — 15 *days*)

Legend	Sex	No. mice	Mean dissemination value	Averaged mean value
Infected, untreated	M	5	4.2	
	F	4	4.0	4.1
Infected, treated daily with 0.2 ml of pregnant mouse serum	M	5	1.0	
	F	5	2.2	1.6

t 4.44
Deg. of freedom 17
P . . . . . . ∠ .005

Table 2 records the data for infected male and female mice treated with Gonadogen. The most surprising feature of these results is the fact that a dosage schedule of 0.4 I.U. per mouse per day had little if any effect on the severity of the infection for male and female mice if treatment was instituted the day after the animals were infected, but this same dosage when instituted 15 days after infection significantly enhanced the severity of the infection for both sexes. Starting this same treatment schedule the day after infection but doubling the dose every 6 days was no more deleterious to female mice than the schedule of unaltered doses, but in the male animals there was a significant increase in the mean dissemination value and the mortality data as compared to those groups treated with the unaltered dosage schedule (compare groups 4–73E to 4–741 and 4–73F to 4–74J in table 2).

The same schedules of treatment used above did not alter the mean dissemination values and mortality data when male and female mice were exposed to a more severe infection (table 2).

TABLE 2. *Effect of Gonadotropin from Pregnant Mare's Serum (Gonadogen) on Mice Infected with Moniliasis (Duration of experiment — 23 days)*

Infected mice treated with Gonadogen	No. Mice	Sex	Mean dissemination value	Mortality (%)
Untreated (4–73A)	30	M	1.0	0.0
0.4 I.U. (0.1 ml) per mouse per day, subcut., treatment started day after infection (4–73E).	30	M	2.1	10.0
Treated as in 4–73E but started 15 days after infection (4–74A)	26	M	5.0	34.6
Treated as in 4–73E but dose doubled every 6 days (4–74I)	30	M	4.3	23.4
Untreated (4–73B)	30	F	1.4	3.3
Treated as in 4–73E (4–73F)	30	F	1.4	3.3
Treated as in 4–74A (4–74B)	26	F	4.3	26.9
Treated as in 4–74I (4–74J)	30	F	1.3	3.3
Infected with mucin adjuvant, untreated (4–73C).	30	M	8.7	50.0
Infected as in 4–73C, treated as in 4–73E (4–73G). . . .	28	M	8.8	46.4
Infected as in 4–73C, treated as in 4–74A (4–74C) . . .	59	M	8.9	50.9
Infected as in 4–73C, untreated (4–73D)	30	F	8.6	46.7
Infected as in 4–73C, treated as in 4–73E (4–73H) . . .	30	F	8.4	46.7
Infected as in 4–73C, treated as in 4–74A (4–74D).	59	F	7.0	45.8

Table 3 is a duplicate of the experiment described in Table 2 except that the dose of Gonadogen was increased to 1.6 I.U. per mouse per day. It can be seen that this increase in dosage enhanced the severity of the infection for male and female mice whether treatment was instituted the day after infection or 15 days afterward. Increasing the dose of Gonadogen still further by doubling

TABLE 3. *Effect of Gonadotropin from Pregnant Mare's Serum (Gonadogen) on Mice Infected with Moniliasis (Duration of experiment — 23 days)*

Infected mice treated with Gonadogen	No. mice	Sex	Mean dissemination value	Mortality (%)
Untreated (4–82A)	30	M	2.2	20.0
1.6 I.U. (0.1 ml) per mouse per day, subcut., treatment started day after infection (4–82E)	60	M	6.5	35.0
Treated as in 4–82E but treatment started 15 days after infection (4–82M).	58	M	4.1	29.3
Treated as in 4–82E but dose doubled every 6 days (4–83H)	30	M	11.3	60.0
Untreated (4–82B)	30	F	3.2	20.0
Treated as in 4–82E (4–82F)	60	F	6.4	33.3
Treated as in 4–82E but treatment started 15 days after infection (4–83A)	60	F	4.4	23.4
Treated as in 4–82E but dose doubled every 6 days (4–83I)	30	F	9.4	46.7
Infected with mucin adjuvant, untreated (4–82C).	30	M	23.2	100.0
Infected as in 4–82C, treated as in 4–82E (4–82G) . . .	60	M	21.1	93.3
Infected as in 4–82C, treated as in 4–82M (4–83B) . . .	60	M	21.8	95.0
Infected as in 4–82C, treated as in 4–82E but dose doubled every 6 days (4–83J) . . .	29	M	15.0	62.1
Infected as in 4–82C, untreated (4–82D)	30	F	25.9	100.0
Infected as in 4–82C, treated as in 4–82E (4–82H)	60	F	22.2	91.8
Infected as in 4–82C, treated as in 4–82M (4–83C) . . .	60	F	24.7	90.0
Infected as in 4–82C, treated as in 4–82E but dose doubled every 6 days (4–83K). . .	30	F	9.1	53.3
Infected as in 4–82C, treated with 0.4 I.U. (0.1 ml) Gonadogen per mouse per day, subcut., dose doubled every 6 days (4–83L).	30	M	15.6	66.7
Infected as in 4–82C, treated as in 4–83L (4–83M) . . .	30	F	14.0	60.0

the dose every 6 days enhanced the mean dissemination values and mortalities for male and female mice to a still greater degree. However, these results apply only to animals receiving an infection of rather low severity. Male and female mice infected with *C. albicans* suspended in mucin showed no alteration in the severity of the infection except in those groups in which the dosage of Gona-

TABLE 4. *The Effect of Gonadotropin from Human Pregnancy Urine (Pranturon) on Experimental Moniliasis in Mice (Duration of experiment — 25 days)*

Legend1)	Sex	Mean dissemination value	Mortality (%)
Infected, untreated (4–13B). . . .	M	20.5	70.0
Infected, treated with 0.8 I.U. (0.1 ml) intramuscularly (4–13D) . .	M	19.8	70.0
Infected, treated with 1.6 I.U. (0.2 ml) intramuscularly (4–13F) . .	M	23.9	90.0
Uninfected, treated with 1.6 I.U. (0.2ml) intramuscularly (4–13H)	M	4.0	20.0
Infected, untreated (4–13I). . . .	F	19.5	70.0
Infected, treated as in 4–13D (4–13L)	F	3.3	20.0
Infected, treated as in 4–13F (4–13N)	F	17.0	60.0
Uninfected, treated as in 4–13F (4–13P).	F	0.0	0.0
Infected, treated with 0.8 I.U. (0.1 ml) subcut. (4–14B)	M	20.6	90.0
Infected, treated with 1.6 I.U. (0.2 ml) subcut. (4–14D)	M	20.0	80.0
Infected, treated with 0.8 I.U. (0.1 ml) subcut. (4–14H)	F	22.6	80.0
Infected, treated with 1.6 I.U. (0.2 ml) subcut. (4–14J)	F	24.6	90.0
Uninfected, treated as in 4–14J (4–14E).	M	4.7	20.0
Uninfected, treated as in 4–14J (4–14K).	F	3.3	20.0
Uninfected, inoculated solely with mucin adjuvant (4–13G)	M	2.6	10.0
Uninfected, inoculated solely with mucin adjuvant (4–13O)	F	2.2	10.0
t comparing 4–13I to 4–13L			3.6
P			.01–.001

1) Treatment with Pranturon in all cases instituted 2 days after infection using the 5 per cent mucin adjuvant and repeated daily for the duration of the experiment. Ten mice constitute an experimental group in every case.

dogen was doubled every 6 days (note groups 4–83J and 4–83K in table 3). In addition, 0.4 I.U. dosage similarly doubled every 6 days was just as effective in reducing the severity of this infection as the higher concentration (4–83L and 4–83M in table 3).

Table 4 records the data for an experiment similar to those described above except that gonadotropin from human pregnancy urine (Pranturon) was used. Treatment was given by two different routes, intramuscular and subcutaneous, and the only discernable difference was that female mice treated intramuscularly with 0.8 I.U. daily showed a significant reduction in the mortality data and mean dissemination value. This was the only group thus affected in this entire series; otherwise the dosage in either sex used had no effect on the course of the disease in either sex or by either route of injection. This negative activity of Pranturon (table 4) is in accord with the data on Gonadogen (table 3) when comparisons are

TABLE 5. *The Effect of Gonadotropin from Pregnant Mare Serum (Anteron) on Experimental Moniliasis in Mice (Duration of experiment — 29 days)*

Legend[1])	Sex	Mean dissemination value	Mortality (%)
Infected, untreated (3–240D) . . .	M	10.4	80.0
Infected, treated (3–240F)	M	11.6	30.0
Infected, untreated (3–239D) . . .	F	5.1	10.0
Infected, treated (3–239F)	F	3.1	10.0
Uninfected, treated as above (3–219E)	M	0.0	0.0
Uninfected, treated as above (3–239H)	F	0.0	0.0
Infected with mucin adjuvant, untreated (3–240E).	M	16.0	70.0
Infected as in 3–240E, treated (3–240G)	M	8.1	30.0
Infected as in 3–240E, untreated (3–239E)	F	18.0	60.0
Infected as in 3–240E, treated (3–239G)	F	10.2	50.0
Uninfected, inoculated with mucin adjuvant only (4–239I).	F	0.0	0.0
t comparing groups 3–240E and 3.240G			2.86
P			.02–.01

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[1]) Treatment with Anteron in all cases was instituted 2 days after infection and repeated daily for the duration of the experiment. Treatment in all cases consisted of the intramuscular inoculation of 0.8 I.U. (0.1 ml) of the Anteron. Ten mice constitute an experimental group.

made between animals similarly infected and treated with the same dosage of I.U. and by the same route of inoculation.

Table 5 presents the data for gonadotropin from pregnant mare serum (Anteron). Male and female mice receiving mild to moderate infection (3–240D and 3–239D in table 5) were apparently unaffected by a dose of 0.8 I.U. per mouse per day of Anteron. This is in accord with the findings for Gonadogen using one-half this dose (table 2) and also with the data for Gonadogen using twice this dose (table 3). Increasing the severity of the infection (3–240E) and 3–239E in table 5) and retaining this same dosage resulted in a significant decrease in the severity of the infection for males only (3–240G in table 5). This finding is in direct contrast to that found in table 4 for mice having a comparable severity of infection and being treated with Pranturon by the same route and in equal dosage. This difference in results between the two gonadotropins might well be a reflection of the differences in their physiological activity.

Discussion

Some of the provocative features of the data presented here are the ways in which the activity of the gonadotropic hormones, under these conditions, parallels many of the findings previously described for cortisone. For example:

1. The severity of the infection plays a major role in the course of the disease being treated with gonadotropins. Thus, a mild infection becomes more severe under a certain treatment schedule with Gonadogen but an infection of moderate severity is unaltered (table 2). Also, as shown in table 3, a certain regimen of Gonadogen increases the severity of a mild infection but is beneficial to animals with a severe infection. Similar results are also evident from the data of table 5, especially for the male animals.

The role of the severity of the infection of moniliasis in mice being treated with cortisone was shown in previous studies (SCHERR, 1953c) to essentially parallel to these results in that, as a rule, "cortisone . . . enhanced the severity of a mild monilial infection, did not alter the severity of a moderate infection, and significantly reduced the severity of a severe infection".

2. The sex of the animal also plays a major role in these results. Infected female mice are unaffected, at least as manifest by the severity of the infection, when treated with periodic increments of Gonadogen (4–74J in table 2), whereas male animals similarly infected and treated suffered an enhanced dissemination (4–74I). On the other hand, an increased dosage schedule of Gonadogen (and also a different severity of infection) apparently did not affect infected male mice (4–13D in table 4) but resulted in a significant reduction in the severity of the infection of female mice similarly infected and treated (4–13L in table 4). This effect disappears if the animals are treated by another route of inoculation (note groups 4–14B and 4–14H in table 4).

In experiments with cortisone SCHERR (1953b) reported that male mice were more susceptible to treatment with cortisone than female mice.

3. The dosage affects the severity of the infection. This is clearly shown in table 3; male and female mice treated as in groups 4–82G and 4–82H showed no alteration in the severity of the infection, whereas increasing the dosage as in 4–83J and 4–83K significantly reduced the mean dissemination value and mortality. A similar observation was reported for cortisone by ROBINSON *et al.* (1953), who showed that a particular severity of infection with *D. pneumoniae* would be increased, decreased, or go unaltered depending upon the dosage of cortisone used.

Any one of the above three observations in itself is little support for a relationship between cortisone and gonadotropins in explaining some of the properties of cortisone, at least as it affects certain infectious diseases. Taken collectively they warrant more refined studies especially in the light of reports such as that of SOHVAL and SOFFER (1951) indicating that treatment with cortisone may increase the titer of urinary gonadotropins.

The increase in the severity of the infection for both sexes when treatment was delayed 15 days as compared to those, animals in which this same treatment schedule was instituted the day after infection is very puzzling (table 2). The many possible explanations which come to mind would be mere speculation at this stage, but the phenomenon does point out the oftentime overlooked role of varying dosage schedules. In line with this effect is the report of DUFFY and MORGAN (1951), who showed that the effect of cortisone may reduce or enhance the febrile response in rabbits injected with *Shigella dysenteriae* endotoxin depending upon the time-dose relationship used.

The fact that the results described here may vary so widely from one experiment to another merely reflects the influence of many factors (sex, dosage, severity of infection, environmental temperature, etc.) on the course of the infection under the conditions used here.

The results described here are in accord with our previous precept (SCHERR, 1954) that it should be feasible to influence the course of and even actively suppress certain infectious diseases by the use of the proper kind(s), concentrations, and combinations of certain hormones. In line with this thesis, GHOSH and DEY (1945) obtained some favorable results with the use of gonadotropic hormones to treat tinea capitis in children, female children responding better than males. YANEZ *et al.* (1941) got very favorable results in the treatment of mycotic scalp infections with various sex hormones. Extrapolation of these results to other clinical entities must await further experimental studies.

Summary

Various gonadotropic hormones were inoculated into mice infected with systemic moniliasis. It was found that the course of the infection could be modified in a beneficial or deleterious manner depending on such conditions as the severity of the infection, sex of animal, doses used and their schedule of inoculation, route of inoculation and source of gonadotropin. The relationship between these data and their similarity to comparable effects ascribed to cortisone is presented and discussed.

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LIBRI NOVI

R. C. ZAPATER: *,,El Diagnóstico Micológico de Laboratorio''*, Edit. ,,El Ateneo'', Buenos Aires 1956, 161 pages, 54 figures, size 20 × 14 cm, $ 2.

For several years there has been a constant demand for books on medical mycology in which the pathogenic fungi are arranged in a taxonomical order. ZAPATER tries to cope with this demand by offering a very handy, richly illustrated, small volume. Chapter I of this booklet deals with general aspects and with the distribution and frequency of the mycoses in Argentina. Chapter II is a short introduction to the knowledge of the morphology of the pathogenic fungi. The necessary mycological techniques are described in chapter III. Chapter IV deals with taxonomy. A dichotomical key for the identification precedes the part in which the pathogenic fungi and actinomycetes are arranged in a taxonomical system. The mycoses are described in the following 25 chapters, the dermatomycoses requiring 8 chapters. Problems concerning fungus allergy are discussed in the last chapter. There are bibliographies at the end of most of the chapters and the formulae of the principal media are assembled in an appendix. Included can be found an index of the contents together with a list of the illustrations at the beginning of the book and an alphabetical index at the end.

The text is clear and concise and there are few misprints. A striking one is the misprint of "Trichosporom" instead of "Trichosporon" in all cases except in the index.

That actidione should not be added to the medium for the isolation of Cryptococcus neoformans has not been mentioned. Unfortunately the dubious name Hormodendrum has been used again as a genus name.

The illustrations on the whole are of a fairly good quality. The photo of the chlamydospores of Candida albicans (fig. 17), however, does not give a clear view of these very characteristic spores of this important yeast. In the colour plate fig. 37 there is no alphabetical reference to the text below. This should be corrected in the next edition. Trichophyton mentagrophytes var. persicolor and Trichophyton rubrum are indistinguishable in this figure, while Trichophyton megnini is represented too pale. This small book, written in Spanish, is with its taxonomical treatment of the pathogenic fungi very appropriate for all Spanish reading students who wish to study medical mycology from a not too expensive handy book.

G. A. DE VRIES

Littman, M. L. and Zimmerman, Lorenz E. *Cryptococcosis* (*Torulosis or European Blastomycosis*) pp. 205. Price: $ 8.50. Grune and Stratton, 1956.

The purpose of this volume is the comprehensive presentation of the available information concerning cryptococcosis in a style which combines the features of a monograph and an atlas. The latter is accomplished by 82 black-and-white photographs and four pages which contain 33 full color reproductions. All are of the highest quality. They depict the outstanding clinical features, the gross and microscopic pathology of cryptococcosis and the appearance of C. neoformans in culture media. Most of the material presented is drawn from the unparalleled collection of the Armed Forces Institute of Pathology. The authors, members of the staffs of the Mount Sinai Hospital of New York and of the Armed Forces Institute of Pathology, respectively, write succinctly. They point out that even though the first case of cryptococcosis was reported in 1894 and the first instance of central nervous system involvement twenty years later, at least eighty per cent of the reported cases have been published since 1937. In the discussion of the clinical aspects the often neglected point is emphasized that even though C. neoformans has a great affinity for the central nervous system, cryptococcosis is primarily a pulmonary disease and involvement of other organs is usually secondary. The review of therapy is complete and up-to-date. Drug therapy is still at the stage of the screening of a wide variety of substances for trial. The best chance for cure lies in radical surgical removal of the pulmonary disease before dissemination occurs. One half of the text is devoted to the pathologic, immunologic and mycological aspects of cryptococcosis. Detailed instructions regarding recommended technics for culturing C. neoformans and for its histologic demonstration are appended. A bibliography of 504 references and a thorough index conclude the volume.

This monograph is recommended to the pathologist, mycologist and clinician alike. It has been announced as the first of a series on the clinical mycoses. It is to be hoped that the succeding volumes achieve the standard established by this one.

Thomas G. Benedek

DA SILVA LACAZ, CARLOS: *Manual de Micologia Medica.* Second edition, 422 pages, 161 figures. Distributors "Liteci", Rua Riachuelo, 275, São Paulo, Brasil, 1956.

When a mycological text requires a second edition within less than one year, it attests to its popularity and probably to the fact that it may fill an existing gap. This is all the more surprising, since the Portuguese language already possessed an outstanding and comprehensive Medical Mycology by FLORIANO P. DE ALMEIDA, 1939. The Latin languages have a number of outstanding monographs in this field, like NEGRONI'S Manual, 1938, in Spanish; REDAELLI'S in Italian; LANGERON and VANBREUSEGHEM'S, 1952 in French, all of them notable for their pronounced originality.

LACAZ' presentation lacks all originality. In its most important part it sounds like a servile inventory of the publications of PECK and SCHWARTZ, of WOLF and WOLF, of CONANT et al., of LEWIS and HOPPER, and a few others. The substantial part of the illustrations is borrowed, with due acknowledgment, from their works.

This manual covers the subject under three general headings. The first part presents the general morphobiologic characteristics of the fungi and the mycoses, in general; the second section deals with all the superficial and deep mycoses, clinically and therapeutically; the last part is devoted to mycologic laboratory techniques.

The author is not a dermatologist and thus, it is understandable that he copied, without any criticism, all the grave errors of his numerous sources, accumulated during the last three decades. For LACAZ "tinea pedis", "onychomycosis", "otomycosis" are still "fungus" infections. "Moniliasis" is everything when a culture yields Candida albicans from any lesion. "Stomatitis angularis" (a manifestation of vitamin B deficiency), "erosio interdigitalis" (a hematogen-endogenous, bacillary manifestation of the permanent endoparasitism) are still represented as "monilia" infections. "Pityrosporon ovale" is still the causative agent of seborrhea capitis. "Levurides", "Moniliids" are also caused by "Monilias", although they are seborrheic dermatitis of hematogen-endogenous, bacillary etiology.

Quite confusing is the mycological nomenclature used in the text. It would have been more preferable and more enlightening for the student to choose one single nomenclature at the author's good judgment and completely forget the rest, since this manual is presumed to serve more the need of beginners in the field than that of advanced students who probably are able to critically approach the subject.

It is natural that the author feels somewhat more on a firm ground in dealing with systemic mycoses, particularly with the South American variety. Here the clinical and mycological presentations are adequate and well illustrated with original material. The technical part is good and exhaustive. The Portuguese, Spanish, Italian, French and above all, the North American Literature is well selected and grouped under the headings of journals, manuals, and specially arranged under the specific disease entities. However, the modern German mycologic literature hardly gets any consideration.

Manuals and texts are more often than not already outdated at the very moment of their publication by new materials, new facts and ideas carried by the only living literature, the journal literature. This manual, in its second part, is decades behind the facts, and new theories put down in the living journal literature. Writers of manuals, texts have the obligation, if they have the courage and methodological knowledge, to critically approach and present the subject, they deal with. To try to please everybody is the greatest disservice to progress in any field of human knowledge. This manual wholly proves this point.

However, the frame work of this manual is good. To make it a useful book, the second part should be thoroughly revised and rewritten at a future date. In its present form it only disseminates innumerable grave errors, and obvious fallacies and it is destined to cause endless confusion. The Portuguese student definitely deserves something better and more substantial.

TIBOR BENEDEK

GRAY, E. A.: *Microbiologie*, translated from English by A. BOISCHOT, X-222 pages, 14×22 cm, with 25 Figures. Paper. Dunod, Paris, 1956.

This unpretentious booklet grew out of lectures given by the author in courses for laboratory technicians. It covers in eleven chapters the whole field of microbiology on a simple, introductory level. The text is well written, the translation is good. However, in the pages on mycology gross errors mar the presentation. "Yeasts", in general terms, are identified as "Shizosaccharomyces" (sic). It is stated that favus is caused by Trichophyton and Microsporum fungi; that Achorion Schoenleini is the causative agent of favus in cats (!) and very infectious for man and rodents. Phycomycetes are mentioned to cause upper respiratory infections. However, there is no member of this class pathogenic for man. Aspergillus might be a secondary invader but never a primary causative agent of human pneumonomycosis. These inaccuracies detract a great deal from the usefulness of the text and reveal a meager familiarity of the author with medical mycology. The illustrations are satisfactory. Paper and print are good. Index and references are included. Due to the numerous gross errors this book cannot be recommended as an introduction into microbiology.

TIBOR BENEDEK

COUDERT, JEAN: *Guide Pratique de Mycologie Médicale*. 364 pages, 8 tables, 1 synoptic chart. Masson et Cie, Paris, 1955.

After LINDAU'S KRYPTOGAMENFLORA, 1922, in German, NANNIZZI'S REPERTORIO SISTEMATICO DEI MICETI DELL' UOMO E DEGLI ANIMALI, 1934, in Italian, the mycological literature is enriched by the latest determinative key of COUDERT, in French. While the others are purely determinative keys, this work contains more than that. In the three parts of the work the first one gives an excellent and concise technic, useful in medical mycology, such as collection of specimens, direct examination, culture, and methods of diagnostic immunology. The second part deals with the systematic diagnosis of human pathogens. The third part discusses in great detail the systematic description of the Genera and Species.

The text is excellently written, clear and concise. The author is especially qualified for writing this text in a borderline field, such as medical mycology is, because he is not only a mycologist of long experience but also a noted dermatologist. The synoptic chart of the main mycotic syndromes is very useful. The tables depicting the organs of fructification of the dermatophytes are most instructive. The book closes with a special "lexicography", an explanation of common mycological terms. There is also a thorough alphabetical index, often sorely missed in French publications.

Due to the excellence of this determinative key it is hoped that a second edition will be forthcoming not before long. In this event certain dermatologic entities which have nothing to do with fungus infection, like "dishidrosis", "moniliasis", "otomycosis", "onychomycosis" should be definitely eliminated as "dermatomycosis". The make-up of the work is superior as to print, paper and illustrations. It can be warmly recommended for the laboratory of dermatomycologists as well as botanists.

Tibor Benedek

ASPERGILLUS GRÁCILIS Bainier VAR. SARTORYI (BIOURGE) BATISTA, LIMA E VITAL N. VAR. - SUA IMPORTÂNCIA FILOGENÉTICA

A. Chaves Batista, [1]
Oswaldo Gonçalves de Lima e [2]
Albino Fernandes Vital [3].
UNIVERSIDADE DO RECIFE — INSTITUTO DE MICOLOGIA Publiçacão no 4[4]

(15.1.1955)

(Com 15 figuras)

O fungo em aprêço apareceu-nos como contaminante de cultura de *Candida stellatoidea* Jones e Martin, despertando a nossa atenção pela morfologia de seus conidioforos, em parte de *Aspergillus* e em parte de *Penicillium.*

Examinado detidamente, permitiu-nos reconhecer que a aparente confusão das suas características era uma constante específica e não uma simples variação, induzida por fatores do meio, como se poderia imaginar, e, muito menos, o produto da associação, num cultivo, de dois fungos distintos.

A análise taxonômica revelou-nos tratar-se, por seu turno, de um *Aspergillus*, do grupo ,,*glaucus*'', na série ,,restrictus'', de Thom e Raper (1945).

A série ,,restrictus'' que compreende as fórmas intermediárias entre os grupos de *A. glaucus* e *A. fumigatus*, — Intermediate Forms — de Thom e Church, (1926), reune, de modo paralelo a todos os *Aspergillus* de aparelho conidial semelhante a *Penicillium*, ou seja o grupo de *A. penicilloides* de G. Smith (1931), tendo *A. restrictus* Smith como tipo.

O conceito de Thom e Raper ao erigir a série ,,*restrictus*'' foi o de que essa série abrangeria os fungos de restricto desenvolvimento cultural e conidios equinulados ou espinescentes, em longas cadeias, compactas, aproximados aos do grupo *A. glaucus*. Dêsse modo, a simples semelhança do aparelho conidial, das chamadas fórmas intermediárias, com *A. glaucus*, os levou, a THOM e RAPER, à nova série „*restrictus*" estimando-a como integrada por elementos anormais de *A. glaucus*, sem tomar em consideração o marcante relevo que tais formas exibem em relação a *Penicillium*, como se verifica de suas palavras „the important relationship indicated by the structure of the conidial apparatus is not with *Penicillium* but with the *A. glaucus* group of which these forms appear to be merely reduced members."

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[1] Chefe do Instituto de Micologia;

[2] Diretor do Instituto de Antibióticos e Prof. de Microbiologia da Escola de Química;

[3] Assistente micologista do Instituto de Micologia — (Todos da Universidade do Recife).

[4] Os autores declinam o seu agradecimento ao Dr. Heraldo da Silva maia, Assistente micologista e D. Marilene Maranhão Moreira, Auxiliar-Técnico, do Instituto de Micologia, pela colaboração que lhes prestaram, durante a realização do presente trabalho.

Essa conceituação, porém, pondo por terra os *Microaspergillus* de WEHMER e o grupo *A. penicilloides* de SMITH, ao nosso ver, não pode ser tomada como definitiva.

Faltam-lhe os limites naturais necessários para uma base nomenclatural sólida, desprezada, como foi, a característica fundamental da morfologia dos conidioforos, e contestada a sua profunda similitude para com *Penicillium*.

O comodismo didático que preocupou aos autores citados, na criação de série „restrictus" terá de ceder terreno à evidência dos fatos, restringindo-se mais ainda o grupo artificial de *A. glaucus*, e situando-se, então, a série de *A. restrictus* como intermediária entre os gêneros *Aspergillus* e *Penicillium*, até que novos estudos digam da conveniência ou não de se restaurar *Microaspergillus*, como ponte entre êsses dois gêneros.

No caso presente, o *A. gracilis sartoryi* n. var. afigura-se-nos como de extraordinária importância, podendo mesmo ocupar a posição de elemento de transição filogenética entre *Aspergillus* e *Penicillium*, uma vez que a morfologia de sua frutificação conidial apresenta caracteres dêsses dois gêneros. Fig. 1-b-c.

O alicerce da taxonomia micológica repousando ainda em grande parte, classicamente, no estudo da morfologia das estruturas vegetativas e de reprodução dos fungos, reforça-se para a diferenciação das espécies de *Aspergillus* e *Penicillium* no estudo do seu comportamento cultural em diferentes meios de laboratório, enquanto não se faz sensível a contribuição da Genetica, no esclarecimento da potencialidade cromozômica dos organismos pertencentes a êsses generos.

Assim, levando em conta o tipo dos conidioforos que se erguem do substrato, com a presença de celula pé (foot cell) Fig. 1-a, próprios de *Aspergillus*, e os conidioforos que se definem sôbre cordões micelianos aereos, (ropes), com vesículas disfarçadas em simples dilatações e esterigmas inseridos ao modo das frutificações monoverticiladas de *Penicillium*, ou ainda proliferantes, com a produção de novas pseudo-vesículas, mantendo, o aspecto geral das ramificações de *Penicillium*, não podemos deixar à margem, em função embora que sómente da morfologia, êsse caráter de conexão entre os dois generos *Aspergillus* e *Penicillium* que o *A. gracilis sartoryi* n. var. bem representa.

Achamos mesmo, ante os resultados de seu cultivo, adiante expostos, que êsse fungo, filogeneticamente, merece uma consideração tôda especial.

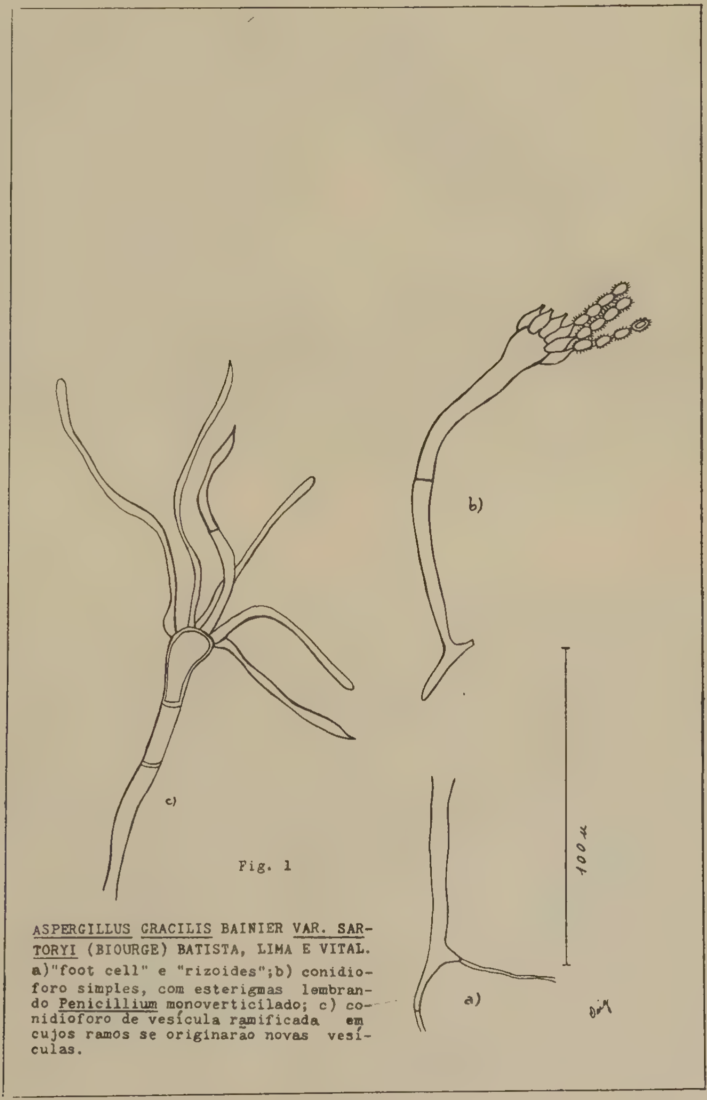

Fig. 1

ASPERGILLUS GRACILIS BAINIER VAR. SARTORYI (BIOURGE) BATISTA, LIMA E VITAL. a)"foot cell" e "rizoides";b) conidioforo simples, com esterigmas lembrando *Penicillium* monoverticilado; c) conidioforo de vesícula ramificada em cujos ramos se originarao novas vesículas.

Fig. 1.

Outrossim, a não formação de cleistotecios e ascosporos, e a ausência de esclerocios que acompanham às culturas dos fungos da série „restrictus" e em particular de *A. gracilis sartoryi*, ressaltam

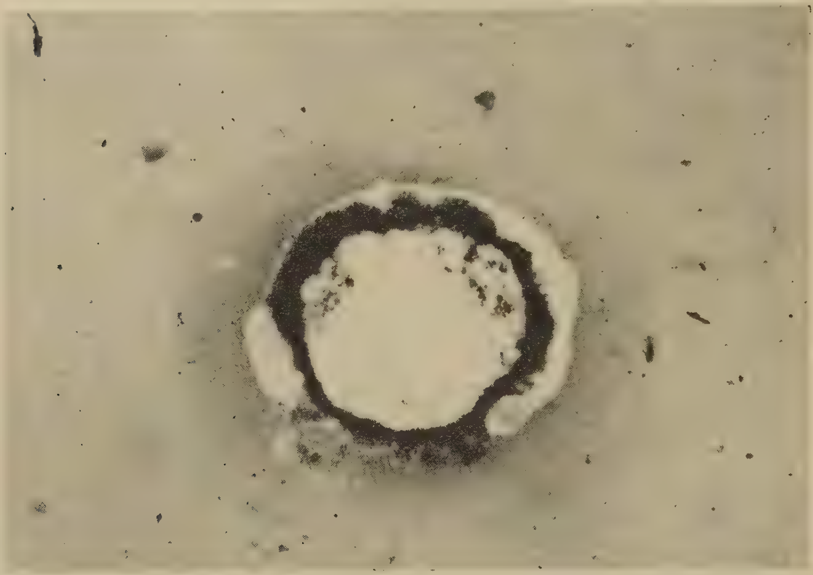

Fig. 2. *Aspergillus Gracilis* Bainier *Var. Sartoryi* (Biourge) Batista, Lima e Vital. Colônia sôbre Czapek neutro, aos 10 dias. Orig.

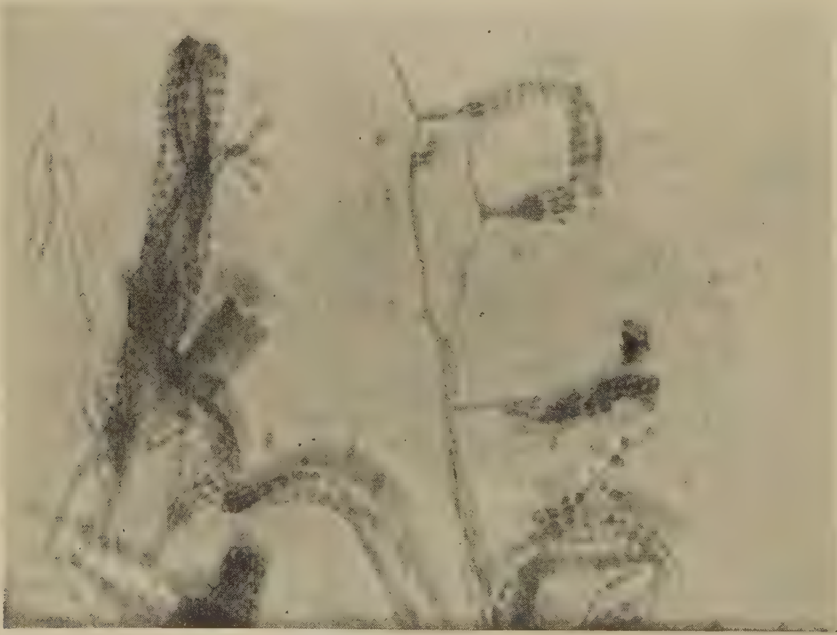

Fig. 3. *Aspergillus Gracilis* Bainier *Var. Sartoryi* (Biourge) Batista, Lima e Vital. Conidioforos simples, desenvolvidos perpendicularmente sôbre cordão miceliano (rope) e cadeias compactas de conidios. Orig. 400 ×.

um estádio incompleto, que se distingue do grupo de *A. glaucus*, geralmente produtor de corpos frutigenos, de natureza sexuada.

Descrição

O fungo *Aspergillus gracilis* Bainier *var. sartoryi* que ora descrevemos apresenta, do ponto de vista da taxonomia, os caracteres gerais que demarcam a espécie *A. gracilis*, e as particularidades que delimitariam *A. sartoryi* Biourge, que, apesar de como tal denominado, não alcançou validade, por falta de publicação adequada, conforme o prescrevem as regras do Código Internacional de Nomenclatura Botânica.

Distanciando-se o nosso fungo de *A. gracilis* pela estrutura do epispório e maior tamanho dos seus conidios poderíamos revalidar a espécie *A. sartoryi* ou descrevê-lo como espécie nova; preferimos, entretanto, reunir o epiteto *sartoryi* ao nome específico de *A. gracilis*, para designa-lo, simplesmente, como uma variedade, numa homenagem a BIOURGE.

No meio de *Czapek-agar*, à temperatura ambiente (26—28° C) e aos 11—12 dias, as colônias revelam-se de lento crescimento, alcançando de 13—16 mms de diâmetro e 1—1,5 mms de altura; são arredondadas, de bordos inteiros ou arborescentes, um tanto mucoides, planas e então de coloração inicial branco-cinza e depois verde cinza com a área central densa, de micelio aereo branco, contrastante, aveludadas, ou se mostram convolutas, ondeadas, de firme textura miceliana, flocosas em parte ou no todo, e de tonalidade branca, com orla estreita verde-cinza, Fig. 2; o micelio submerso ao redor das colônias, origina como que um halo de 1—2 mms de diâmetro; exsudato ausente; odor indistinto ou ausente; reverso, de coloração azul-cinza ou incolor.

Conidioforos de dois tipos: simples e de vesículas ramificadas.

Os conidioforos simples levantam-se do substrato, com celula pé (foot cell) diferençada, ou se constituem a partir de hifas aereas e cordões micelianos (ropes), no sentido perpendicular a êstes, Fig. 3, apresentando-se septados, de membrana lisa, com o ápice clavado ou cônico, e alcançando a extensão de 22,5—110 μ, progressivamente dilatados da base para o ápice, onde o diâmetro varía de 6—12,5 μ.

Os conidioforos de vesículas ramificadas geralmente originam-se do substrato, com ,,foot cell'' ou com ,,rizoides'' 101—276 μ de extensão (ramo primário) e o ápice compõe uma vesícula globosa ou claviforme, 10—19 μ de diâmetro da qual proliferam novos conidioforos, pseudo-conidioforos, de base estreitada e ápice alargado em vesícula claviforme, Fig. 4 e Fig. 5; frequentemente, dessas novas vesículas formam-se novos pseudo-conidioforos, 62—287 × 6—17 μ que dão dilatações vesiculares por seu turno também originárias de novos pseudo-conidioforos, de 62—295 × 6—17 μ, perfazendo assim um total de três expansões de conidioforos e três vesículas Fig. 6 e Fig. 7; certas linhagens ainda produzem uma

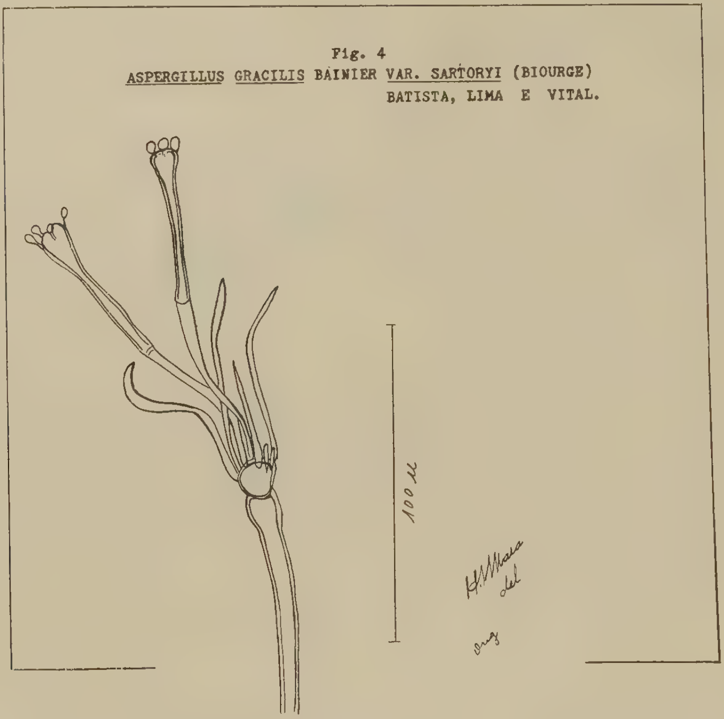

Fig. 4
ASPERGILLUS GRACILIS BAINIER VAR. SARTORYI (BIOURGE)
BATISTA, LIMA E VITAL.

100 μ
H. Massa
del
orig

Fig. 4.

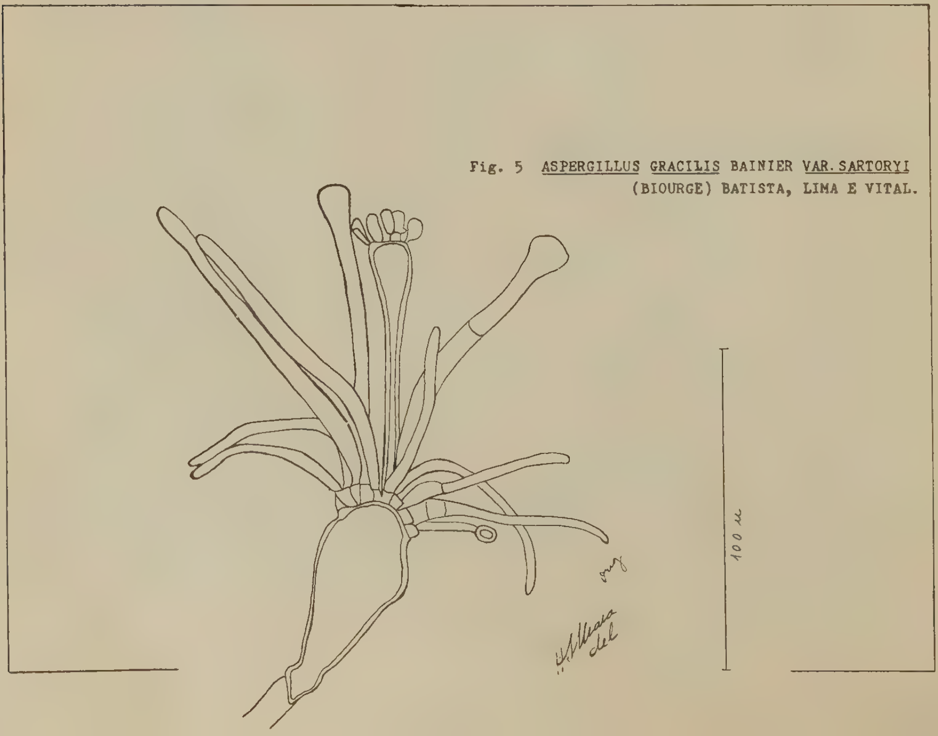

Fig. 5 ASPERGILLUS GRACILIS BAINIER VAR. SARTORYI
(BIOURGE) BATISTA, LIMA E VITAL.

100 μ
orig
H. Massa
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quarta extensão de pseudo-conidioforos encimados também por vesículas.

Os conidioforos simples são abundantes na superfície do meio de CZAPEK enquanto que os conidioforos ramificados se apresentam imersos no meio de cultura, à maneira decumbente, e escassos ou bastante numerosos, Fig. 10.

Nas vesículas dos conidioforos simples inserem-se esterigmas lageniformes, em uma única série, em número de 3 ou em grande quantidade, e sómente na porção mais apical das vesículas, atingindo de 6—9 × 2,5—4 μ.

É comum também o conidioforo depois de entumecer o ápice, na construção de vesícula, dar lugar ao crescimento de longos e disformes esterigmas, assemelhados às ramificações monoverticiladas de *Penicillium*, os quais, entretanto conservam-se estéreis, sem produzir conidios, Fig. 8 e Fig. 9; em outros conidioforos, porém, alguns dêsses esterigmas compõem novas vesículas.

Nos conidioforos de vesículas ramificadas só as vesículas dos últimos pseudo-conidioforos possuem esterigmas, aliás com as mesmas características dos esterigmas das vesículas dos conidioforos simples; as demais não os têm.

Nas vesículas livres, de ramos secundários, os esterigmas suportam curtas cadeias de conidios — Fig. 11, enquanto que as vesículas livres de ramos terciários e quaternários exibem esterigmas com um só conidio ou estereis.

Dos esterigmas dos conidioforos simples partem colunas compactas de conidios, que variam de 90—281 × 17—28 μ, Fig. 12.

Os conidios são em fórma de barril, de tonalidade verde-cinza, 5—7,5 × 4—5 μ, de episporio liso quando jovens, e espinescente à maturidade, ligados uns aos outros por conectivos pouco evidentes, Fig. 13.

No meio de SABOURAUD ,,Difco'' as colônias são mais ampliadas do que sôbre CZAPEK agar chegando a 21 mms de diâmetro, aos 6 dias e à temperatura ambiente; são ondeadas, brancas até o 13° dia, mas em vez de flocosas, mostram-se de aspecto fasciculado, com abundantes cordões micelianos (ropes) à superfície; o micelio, submerso é bem mais reduzido do que em CZAPEK, Fig. 14 e Fig. 15, inodoras, sem exsudato, de reverso branco-cinza; a 37° C o crescimento é paralisado.

Na superfície das colônias encontram-se conidioforos simples ao passo que, imersos no meio de cultura se acham, abundantemente, conidioforos de vesículas ramificadas e até cordões micelianos dos quais se originam conidioforos simples, no sentido perpendicular.

Sôbre papel de filtro assentado na superfície do meio de SABOURAUD, em placas de PETRI, segundo a técnica de FLEMMING, as culturas desenvolvem-se com certa intensidade, chegando a 20 mms de diâmetro, planas ou enrugadas, de coloração branco-creme; tais colônias produzem, indistintamente, tanto sôbre o papel, como entre o papel e o meio de cultivo, conidioforos simples e conidioforos de vesículas ramificadas; algumas linhagens entretanto formam preferentemente os conidioforos de vesículas ramificadas entre o papel e o meio de cultura.

No meio de batata-dextrose-agar as colônias são planas, velutinas, quase sem desenvolvimento de micelio aéreo, e permanecem de coloração verde-cinza.

Os conidioforos simples não se distinguem daqueles que se formam sôbre Czapek-agar.

Os de vesículas ramificadas, originando-se também sôbre ,,foot cell'', ou sôbre ,,rizoides'' apresentam o ramo primário mais longo e mais estreito, 146—300 × 8,5—11 μ; os ramos secundários variam de 62—135 × 8,5—11 μ; não assinalamos ramificações terciárias e quaternárias nas culturas sôbre batata-dextrose-agar.

Em regra os conidioforos simples se acham na superfície das colônias e os conidioforos de vesículas ramificadas estão imersos no meio de cultivo.

As cadeias conidiais oscilam entre 45—140 × 17,5—25 μ e os conidios, individualmente, revelam-se de menor tamanho, 5—6,5 × 3—4 μ.

O fungo em apreciação apresenta clamidosporos globosos ou ovoides, intercalares, em rosário ou isolados, em todos os meios de cultura e à medida de seu envelhecimento.

Não desenvolve esclerocios nem constitui cleistotecios.

Isolado como contaminante de cultura no laboratório.

Leg. A. Fernandes Vital, 8/11/1954.

Typo, no 246, no Instituto de Micologia, da Universidade do Recife.

Aspergillus gracilis Bainier var. *sartoryi* (Biourge) Batista, Lima e Vital.

Coloniae in agaro Czapekii neutro multo restrictae, 13—16 mm *diam., in Sabouraudii dextrosi celeriter crescentes, planae, albido-cinereae, dein viridae — cinereae et area centrali denso-floccosae, albidae, vel convolutae, corrugata compositae, albae et margine viridae in agaro Sabouraudii fasciculatae; mycelio submerso in halo marginato; sporulatione media; exsudato nullo; reverso incolorato vel caeruleo-cinereo, in agaro Sabouraudii dextrosi incolorato vel albo-cinerae, inodora: conidiophoris simplices et conidiophoris vesicula ramosis gerentes.*

In agaro Czapekii conidiophoris simplices cum ,,foot cell'', plerumque ex hyphis repentibus orientibus membrana laevis, septatis, ad apicem clavatis vel conicis, 22,5—110 × 6—12,5 μ *superficiali.*

Conidiophoris vesicula ramosis immersis cum ,,rhizoidis'' vel ,,foot cell'' sed distinctis in ramis; prima ramis 101—276 × 10—19; *vesicula globosa vel claviformia, ramosa; secundarius ramis* 62—287 × 6—17 μ *et vesicula claviformia ipse proliferantibus; tertius ramis* 62—295 × 6—17 μ *et vesicula clavata vel globosa; ramo quarto raro; sterigmatibus in conidiophoris simplicis lageniformibus,* 3 *vel*

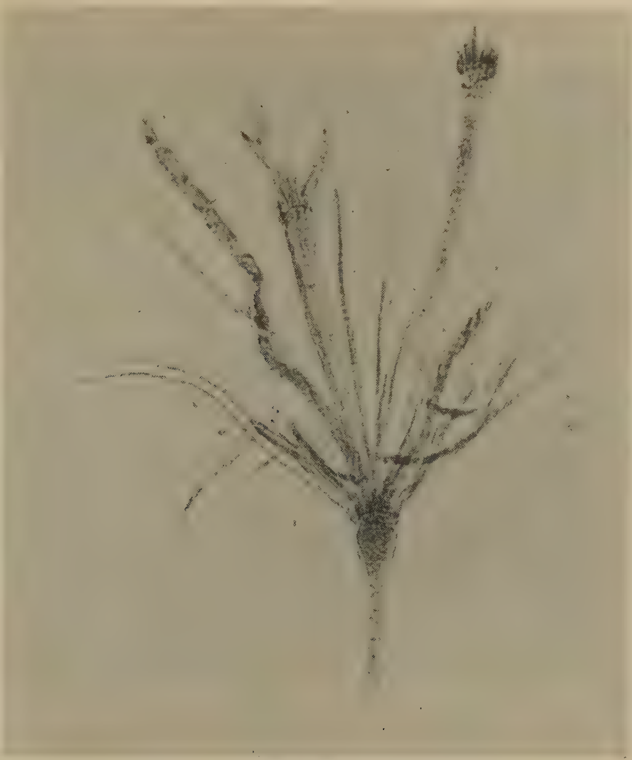

Fig. 6. *Aspergillus Gracilis* Bainier *Var. Sartoryi* (Biourge) Batista, Lima e Vital. Vesícula ramificada, com esterigmas transformados em pseudo-conidioforos que dão novas vesículas. Orig. 400 ×.

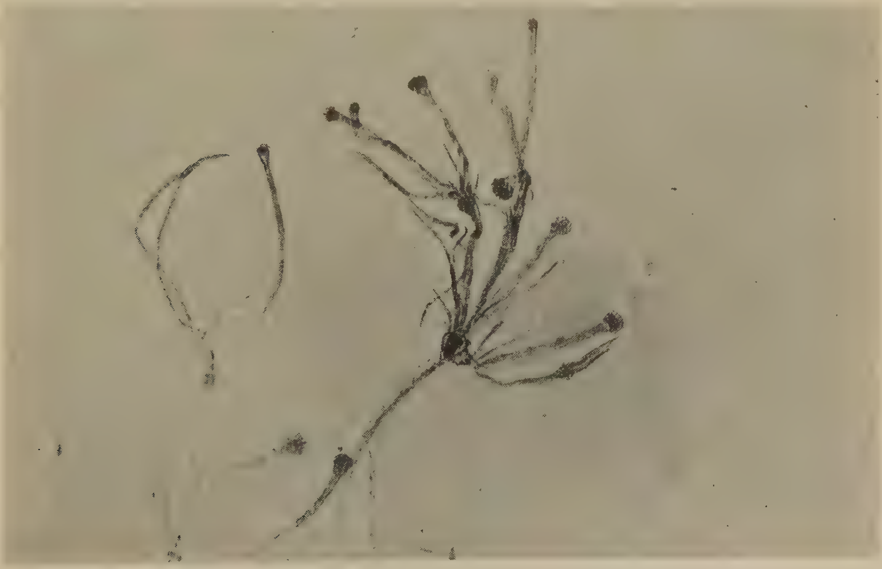

Fig. 7. *Aspergillus Gracilis* Bainier *Var. Sartoryi* (Biourge) Batista, Lima e Vital. Conidioforo (ramo primàrio) vesícula ramosa e pseudo conidioforo (ramos secundàrios e terciarios), os últimos tendo vesículas ferteis, com esterigmas normais onde se insere um só conidio. Orig. 200 ×.

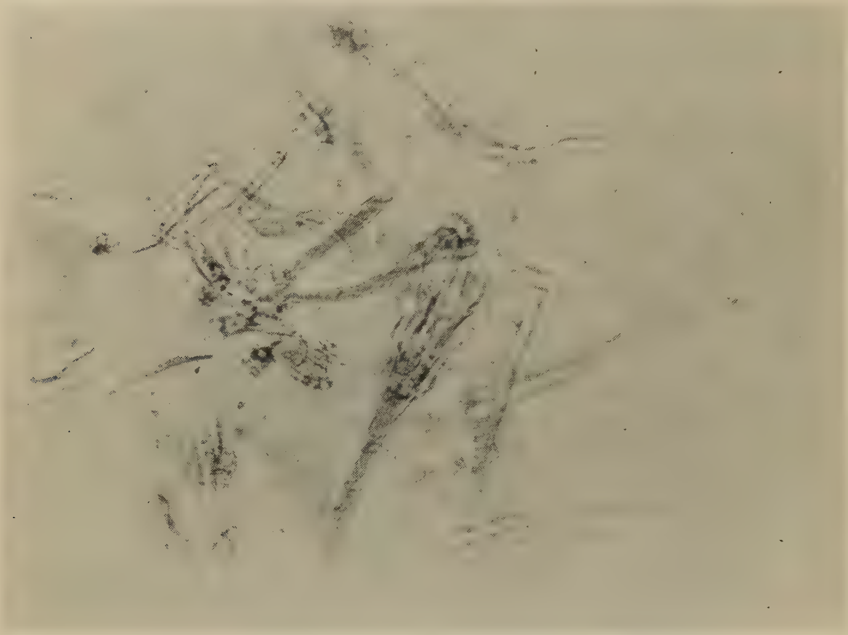

Fig. 8. *Aspergillus Gracilis* Bainier *Var. Sartoryi* (Biourge) Batista, Lima e Vital. Conidioforo com esterigmas uniseriados assemelhando-se à ramificação monoverticilada de *Penicillium*. Orig. 430 ×.

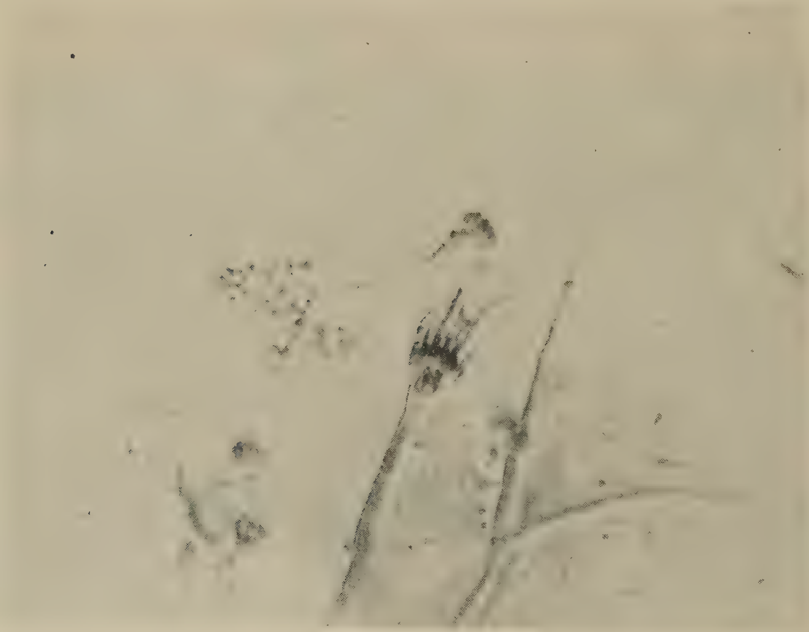

Fig. 9. *Aspergillus Gracilis* Bainier *Var. Sartoryi* (Biourge) Batista, Lima e Vital. Detalhe da inserção de esterigmas anormais no terço apical da vesícula. Foto-micro obtida com filtro vermelho. Orig. 430 ×.

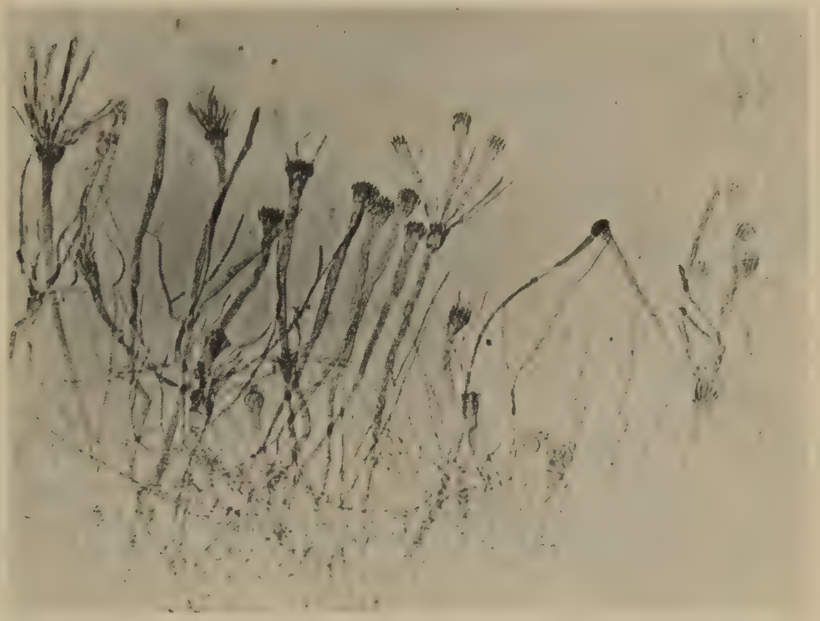

Fig. 10. *Aspergillus Gracilis* Bainier *Var. Sartoryi* (Biourge) Batista, Lima e Vital. Outro aspecto dos conidioforos de vesículas ramificadas, formados na intimidade do meio de Czapek, inteiramente imersos. Orig. 200 ×.

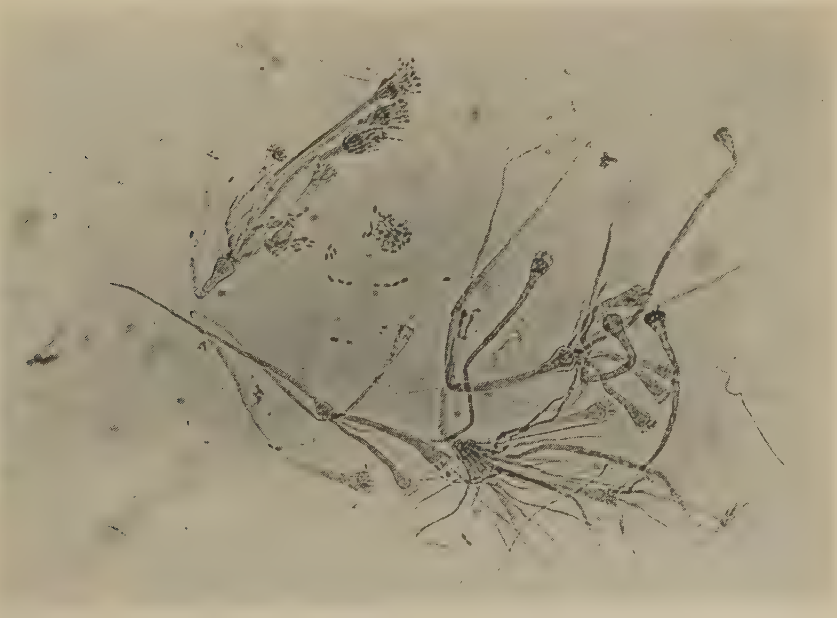

Fig. 11. *Aspergillus Gracilis* Bainier *Var. Sartoryi* (Biourge) Batista, Lima e Vital. Aspecto de conidioforos de vesículas ramificadas, notando-se os ramos primarios e os ramos secundários, êstes com as vesículas sustentando curtas cadeias de conidios. Orig. 200 ×.

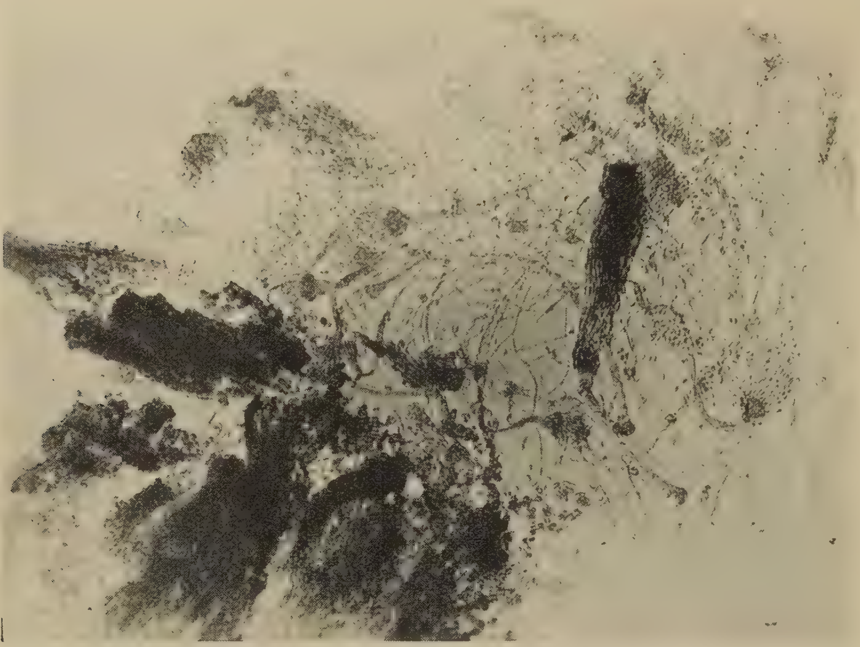

Fig. 12. *Aspergillus Gracilis* Bainier *Var. Sartoryi* (Biourge) Batista, Lima e Vital. Cadeias conidiais compactas, como verdadeiras colunas, desenvolvidas sôbre conidioforos simples, no meio de Czapek. Orig. 200 ×.

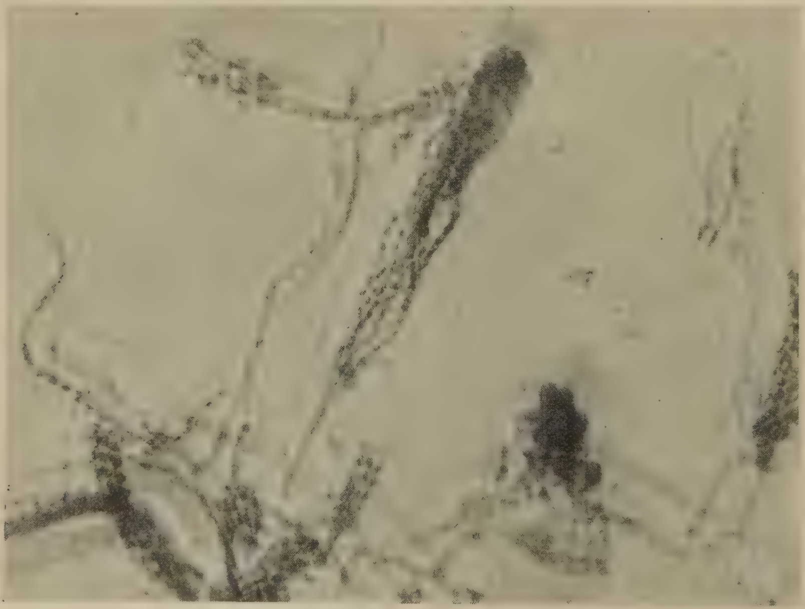

Fig. 13. *Aspergillus Gracilis* Bainier *Var. Sartoryi* (Biourge) Batista, Lima e Vital. Detalhe da constituição das colunas de conidios, sôbre conidioforos simples. Orig. 400 ×.

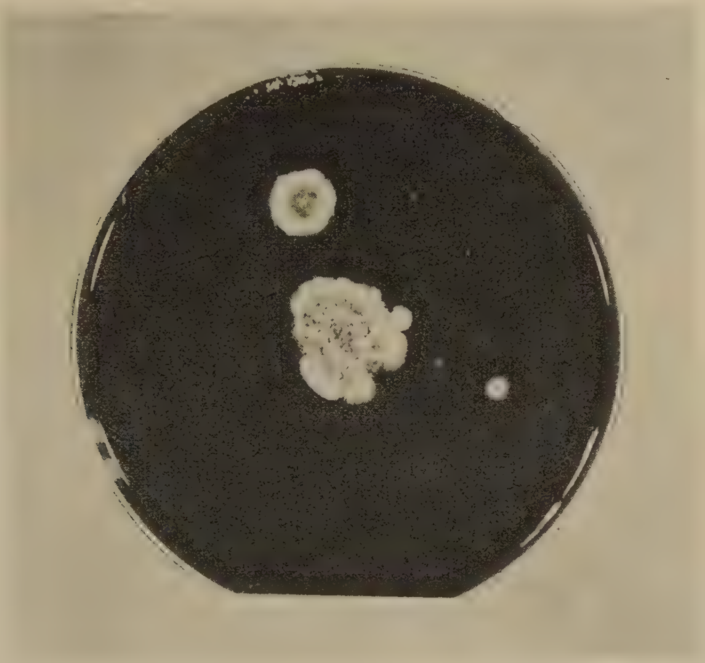

Fig. 14. *Aspergillus Gracilis* Bainier *Var. Sartoryi* (Biourge) Batista, Lima e Vital. Colônias em Sabouraud-dextrose, aos 7 dias. Orig.

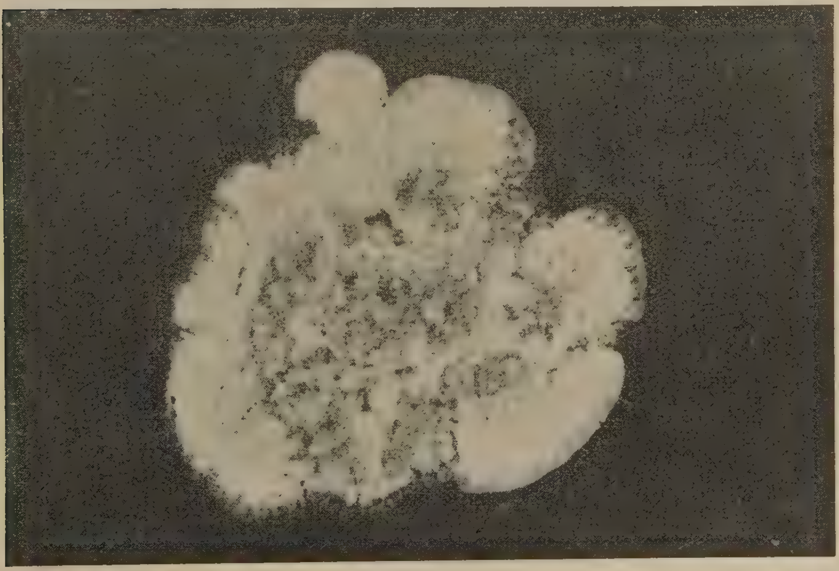

Fig. 15. *Aspergillus Gracilis* Bainier *var. Sartoryi* (Biourge) Batista, Lima e Vital. Detalhe da superfície da cultura, em Sabouraud-dextrose, aos 7 dias. Orig.

numerosis, 6—9 × 2,5—4 μ; *in vesicula ramosis sterigmatibus solum in suprema, dense confertis, cum uno solum conidiis; in sterigmatibus conidiophoris simplicis, conidiis in cadusi, viride-cinereis*, 5—7,5 × 4—5 μ, *conspicuae spinescentis, in catenis longis*, 90—281 × 17—28μ *saepe spissus parallelibus.*

Contaminatione in laboratorio, Recife, Brasil.

Leg. A. F. Vital, 8/11/1954. Typus, 246, Instituto de Micologia, Universidade do Recife.

Prov. Pernambuci, Brasiliae, Amer., Austr.

Summary

We deal with a new variety of *Aspergillus* which we call *Aspergillus gracilis* Bainier *var. sartoryi* (Biourge) Batista, Lima and Vital.

This variety appears to be very important under the phylogenetic point of view, apparently being an intermediate form between *Aspergillus* and *Penicillium.*

Its morphology resembles *A. gracilis* but the conidia characteristics are distinct; we took the epithet *sartoryi* from *A. sartoryi* Biourge which was not described by the respective author.

We suggest, too, the transfer of the series *A. restrictus*, where we put our new variety, from the group *A. glaucus*, to become independant, as a connective series in the phylogenetic development between *Aspergillus* and *Penicillium*, since the type of the series and the other elements that it embraces does not produce sclerotia or cleistothecia and the conidial apparatus offers curious similarity to *Penicillium.* Besides, some fungi of the series grow well in certain culture media, in complete discordance with the members of the group *A. glaucus.*

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ESTUDO TAXONÔMICO DE UM NOVO ASPERGILLACEAE

A. Chaves Batista e Heraldo de Silva Maia [1])
UNIVERSIDADE DO RECIFE — INSTITUTO DE MICOLOGIA, (Publicação No 5)

(15.1.1955)
(Com 18 figuras)

O trabalho em tela focalisa a descrição de um novo *Aspergillaceae* com que vimos lidando de há muito, o qual, ante suas caracteristicas morfológicas, se sitúa entre os organismos de estrutura complexa, por suas diversas fórmas, que são antes de tudo o produto de variação fenotípica, de causa até agora ignorada.

As observações que conseguimos reunir a propósito da diversificação do comportamento dêsse fungo sôbre diferentes meios artificiais de cultura afastam quasi que inteiramente das cogitações, a influência da qualidade do meio, sobretudo a da presença de possiveis substâncias estimulantes, como determinante das modificações do tipo de seus conidioforos, em virtude de os nossos estudos haverem sido conduzidos sempre sôbre os mesmos meios sinteticos, de elaboração industrial.

Algum fator estranho, cuja atuação não nos foi dado notar e, próprio do ambiente ou relacionado com o genoplasma, certamente é o indutor das transformações a que está sujeito o aparelho de frutificação conidial dêsse fungo.

Nenhuma investigação de natureza genetica, porém, logramos executar, para o esclarecimento dessa importante questão; nem conseguimos cleistotecios ou simples órgãos sexuais diferençados, em todos os meios em que o fungo se desenvolveu, que servissem de estímulo a uma pesquisa nesse sentido.

O fato marcante da definição estrutural continuou sendo assim a alteração de seu aspecto morfológico, o fungo apresentando-se ora com conidioforos de vesiculas ramificadas, ora com conidioforos de vesiculas normais, e ainda com conidioforos de vesiculas portadoras de esterigmas transformados em filamentos, como pseudo-conidioforos, estereis, entretanto.

Todas essas modificações ocorrem em qualquer dos meios adiante citados, mas, frequentemente, uma determinada variação se mostra predominante numa certa linhagem e sôbre certo meio de cultivo, para logo desaparecer êsse predomínio, com a transferência do fungo para outra placa de Petri, contendo a mesma qualidade do meio ou meio de natureza distinta.

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[1]) Os autores agradecem a D. Marilene Maranhão Moreira, a colaboração prestada no curso dêste trabalho.

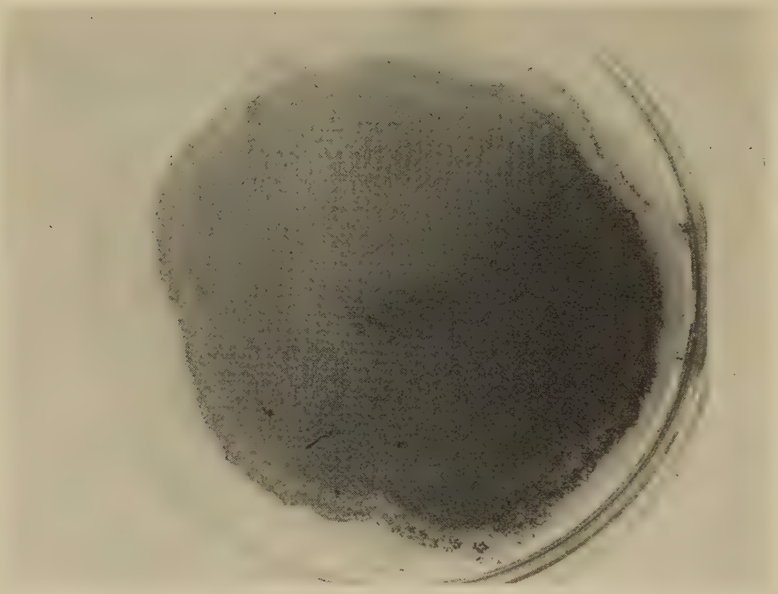

Fig. 1. *Aspergillus fasciculatum* Batista e Maia, n. sp. Colônia, aos 10 dias, em Czapek-agar, neutro. Orig.

A variabilidade da morfologia dos conidioforos aparece como um detalhe de grande significação na taxonomia dêsse fungo, desde quando se faz mistér apreciar a sua ocurrência, previamente, para que possa ter lugar a diagnose específica.

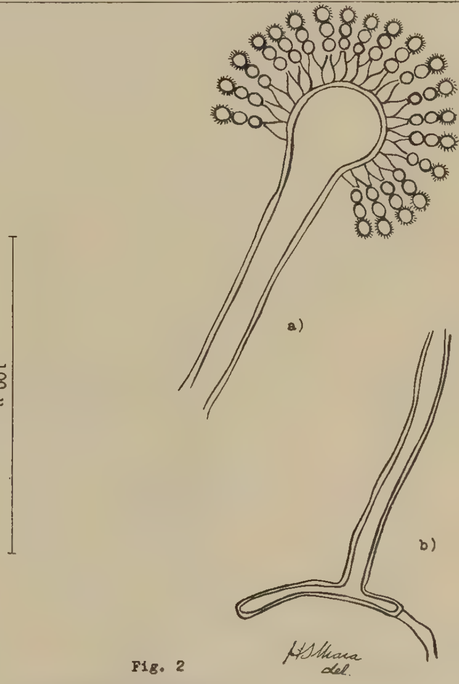

Fig. 2

ASPERGILLUS FASCICULATUM
BATISTA E MAIA.
a) parte de conidioforo de vesícula normal, com esterigmas uniseriados;
b) parte de conidioforo e celula pé.
Orig.

Fig. 2.

O cultivo da *A. fasciculatum* n. sp. sôbre o meio de Czapek-agar-neutro progride rapidamente, alcançando as colonias de 3—9 cms. de diâmetro, aos 10—12 dias e à temperatura ambiente de 26—28°C.

As colonias definem-se como planas, tenues, de micélio vegetativo submerso, hialino, depois aereo, flocoso, inconspicuamente zonadas, com a area central e as margens de tonalidade verde-amarelado, Fig. 1, homogeneamente esporuladas; exsudato e odor ausentes; cleistotecios ausentes; esclerocios produzidos por algumas linhagens, escassos, e não formados por outras; quando presentes são brancos, de comêço, tornando-se depois negros, globoso-deprimidos ou de fórma irregular, e de textura parenquimática; reverso plano, incolor a principio, depois roseo-amarelado, de bordos esverdeados ou de coloração castanho-clara.

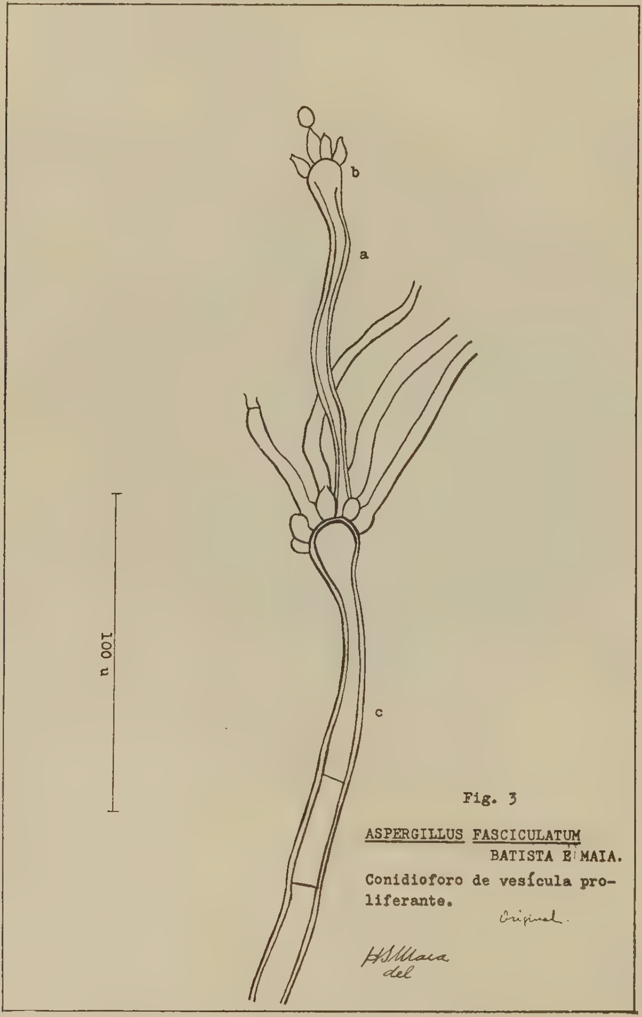
b
a
c
100 u
Fig. 3
ASPERGILLUS FASCICULATUM
BATISTA E MAIA.
Conidioforo de vesícula proliferante.
Original.
HSMaia
del

Fig. 3.

Conidioforos desenvolvidos diretamente do substrato, suportados por celula pé estreita (narrow foot cell), de onde se vão alargando gradualmente, na direção do apice, eretos, de paredes finas, coradas de amarelo ou esverdeadas, ligeiramente-rugosas ou lisas, curtos, de 62,5—175 × 5—7, 5μ nas linhagens (strains) em que a região apical se entumesce em uma só vesícula normal Fig. 2 ou em vesícula proliferante em pseudo-conidioforos Fig. 3, e longos, de 675—1643 × 14—17μ, quando apresentam o apice bifurcado ou com fasciculos de 3 e 4 ramos; em alguns ,,strains'' os conidióforos originam-se tambem de hifas aereas, Fig. 4, b. Os conidióforos são uni ou pluri-septados, ordinariamente bifurcados no apice, Fig. 5, produzindo-se dois ramos que se dilatam em vesiculas globoso-deprimidas, dotadas de 4—8 esterígmas ou de esterigmas em numero indefinido. Fig. 6; às vezes, porém, essas ramificações não chegam a constituir ramos, dilatando-se desde a base, para a formação de vesículas clavadas, Fig. 7.

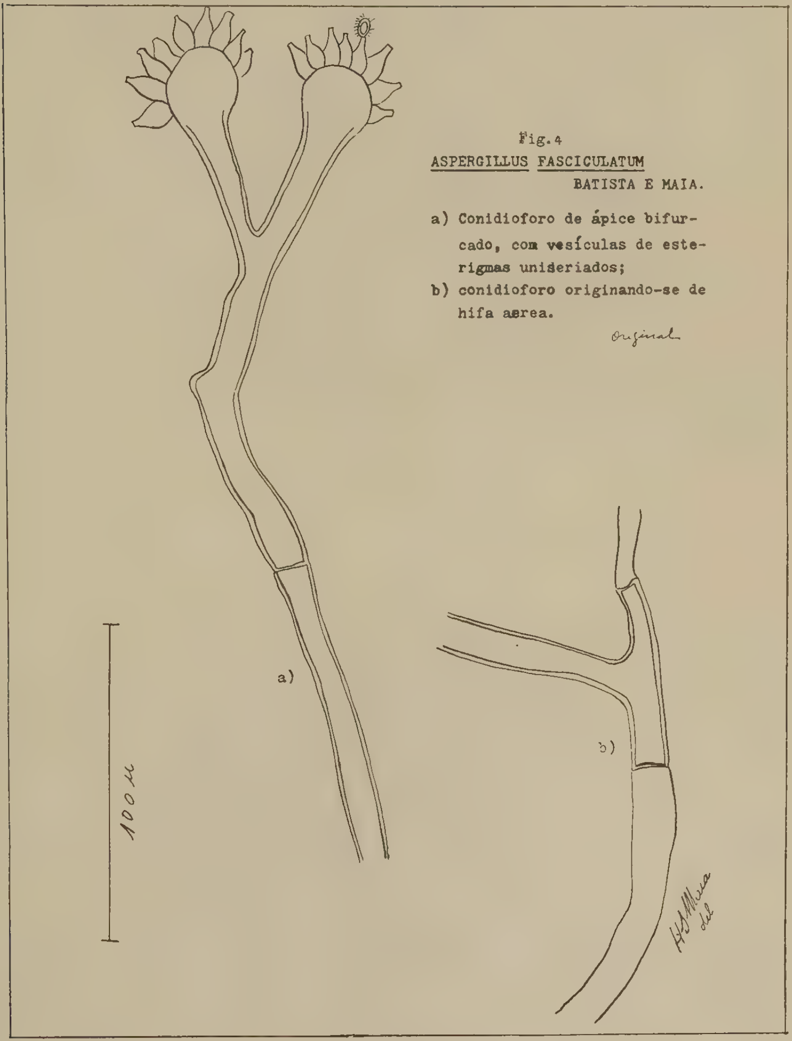
Fig. 4
ASPERGILLUS FASCICULATUM
BATISTA E MAIA.
a) Conidioforo de ápice bifurcado, com vesículas de esterigmas unisseriados;
b) conidioforo originando-se de hifa aerea.
Original
a)
b)
100 µ
HJMaia del

Fig. 4.

Alguns ,,strains'' portam-se quasi caracteristicamente como dotados de conidióforos multiramificados, com ramos em número de 3 e 4, os quais, por seu turno se entumescem na extremidade livre, dando vesiculas subglobosas; essas ramificações derivam da porção terminal do conidióforo, a partir de um septo, Fig. 8 ou aparecem ao longo do conidióforo, Fig. 9, o que empresta assim à frutificação conidial o aspecto fasciculado Fig. 10, que deu o nome à espécie do fungo.

As vesiculas dos conidióforos indivisos, isto é, não ramificados, apresentam-se com uma única série de esterígmas, Fig. 2a, de disposição intrincada, Fig. 12, ou têm duas séries de esterígmas Fig. 11, assemelhando-se, nêsse caso, a *Aspergillus-flavus* Link; são globosas, subglobosas ou conoides, (dome like), hialinas ou levemente esverdeadas, de paredes finas, inteiramente ferteis, 34—67,5 × 22,5—67, 5μ de diâmetro, quando dotadas de esterígmas biseriados; os esterígmas primários são cuneiformes, radiantes, hialinos, de 7,8—17 × 5,5—7, 8μ; os esterígmas secundários, em número de 2—3 para cada esterígma primário, mostram-se lageniformes, de 7,8—11 × 3,3—4,5 μ.

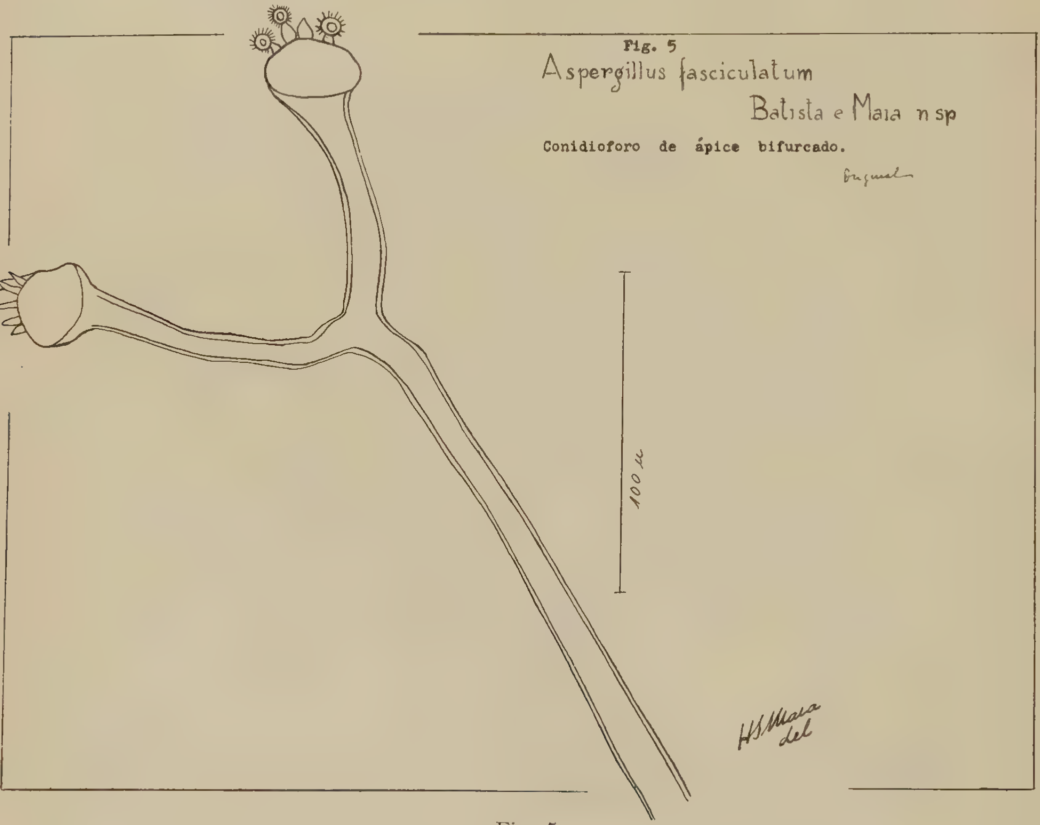

Fig. 5

As vesiculas de conidióforos ramificados, globoso-deprimidas ou truncado-cônicas, de paredes hialinas ou amareladas, delicadas, só em parte férteis, medem de 16—21 × 7,8 —15μ, e, conquanto eventualmente se apresentem com duas séries de esterígmas, em geral revelam-se uniseriadas, Fig. 13, com esterígmas lageniformes, hialinos ou amarelados, de 6,7—10 × 3,5—5,6μ.

Ao lado dessa complexa variação morfologica do conidióforo, Fig. 14, algumas linhagens exibem transformações das próprias vesículas, as quais se manifestam especialmente sôbre conidióforos normais, não ramificados, raras vezes ocorrendo igualmente sôbre conidióforos bifurcados ou ramosos.

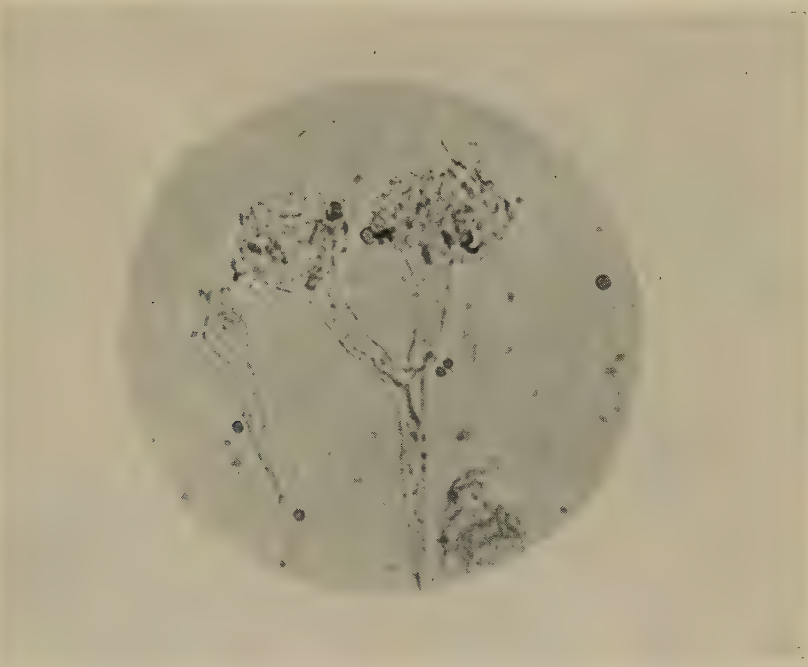

Fig. 6. *Aspergillus fasciculatum* Batista e Maia, n. sp.
Conidioforo não septado apresentando duas vesículas; ao lado esquerdo é visto um conidioforo normal. Orig. 400 ×

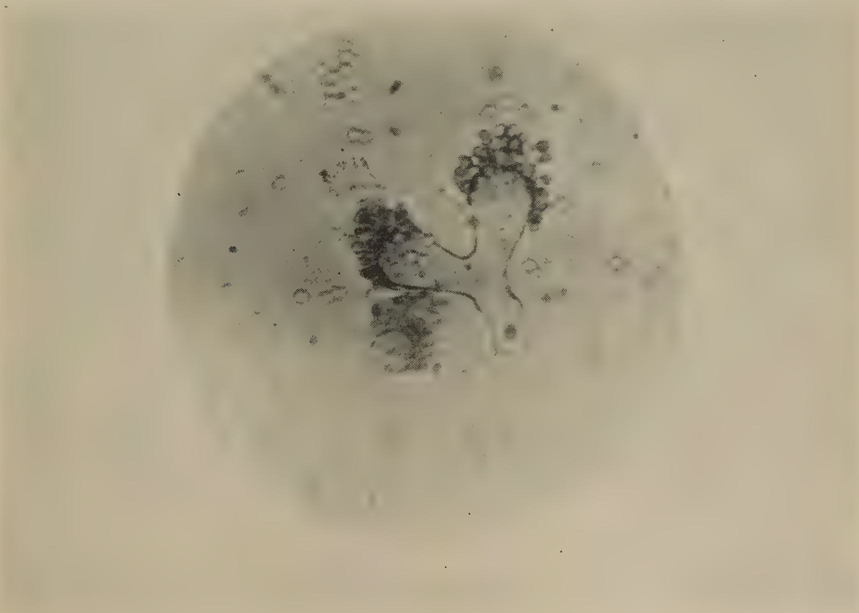

Fig. 7. *Aspergillus fasciculatum* Batista e Maia, n. sp.
Conidioforo bifurcado e bifurcações dilatadas em vesículas uniseriadas, vendo-se ao lado um conidioforo e vesícula normais. Orig. 400 ×.

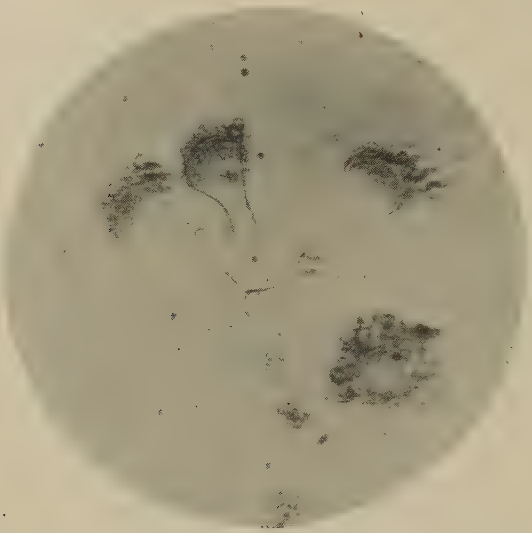

Fig. 8. *Aspergillus fasciculatum* Batista e Maia, n. sp.
Conidioforo com três vesículas inseridas no septo apical, observando-se uma vesicula normal ao lado direito. Orig. 430 ×

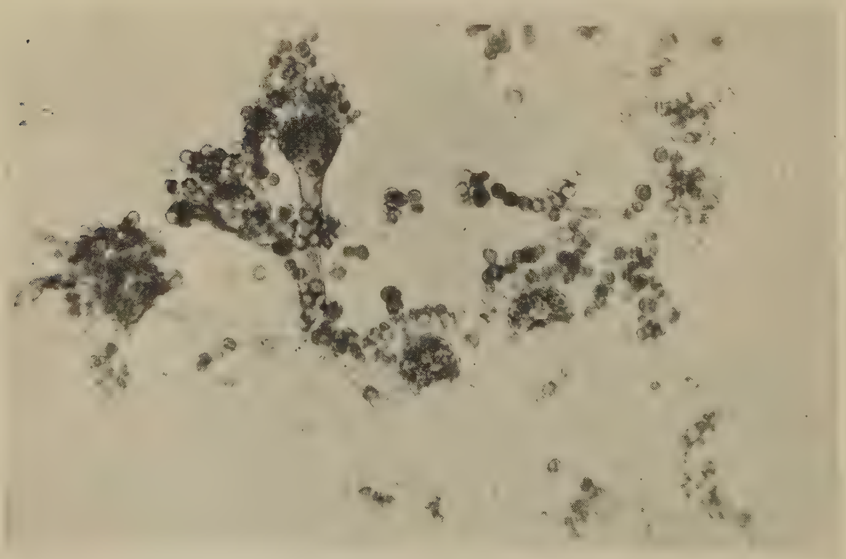

Fig. 9. *Aspergillus fasciculatum* Batista e Maia, n. sp.
Conidióforos bi e trifurcados, no último caso, com os ramos originados lateralmente, sôbre o conidioforo. Orig. 400 ×.

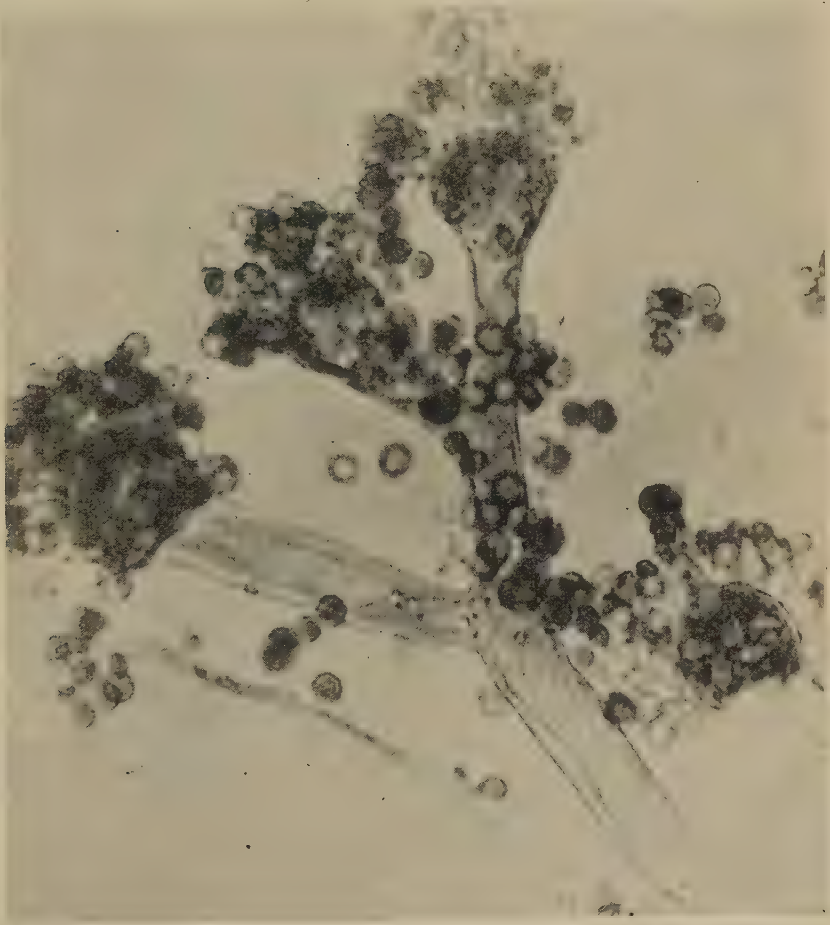

Fig. 10. *Aspergillus fasciculatum* Batista e Maia, n. sp.
Detalhe da ramificação do conidioforo para a produção de "cabeças" anormais, cujo conjunto dá á vegetação do fungo o caráter de frutificação conidial fasciculada. Parte da Fig. 4, 400 ×. Ampliada 2 × Original.

Essas transformações consistem propriamente no alongamento dos esterígmas — esterígmas proliferantes — e na proliferação de esterígmas alongados e de pseudo-conidióforos, êstes com o ápice dilatado em vesícula, à maneira de *Aspergillus gracilis* — Bainier ou de *Aspergillus proliferans* G. Smith.

Excepcionalmente, ao longo de um conidióforo ou mesmo no ápice dêste, desenvolve-se um entumescimento, como se fôra um ,,grampo conectivo'' o qual se individualisa em vesícula lateral, enquanto o conidióforo continua o seu crescimento; essa vesícula possui esterígmas alongados, anormais e pseudo-conidióforos com a porção final constituida em nova vesícula, com uma série de esterígmas, Fig. 15.

São raras as vesículas dotadas simplesmente de esterígmas alongados, como uma indicação de que a tendência deles é para originar vesículas secundárias; tais esterígmas alongados dominam inteiramente a região vesicular fértil, apresentando-se

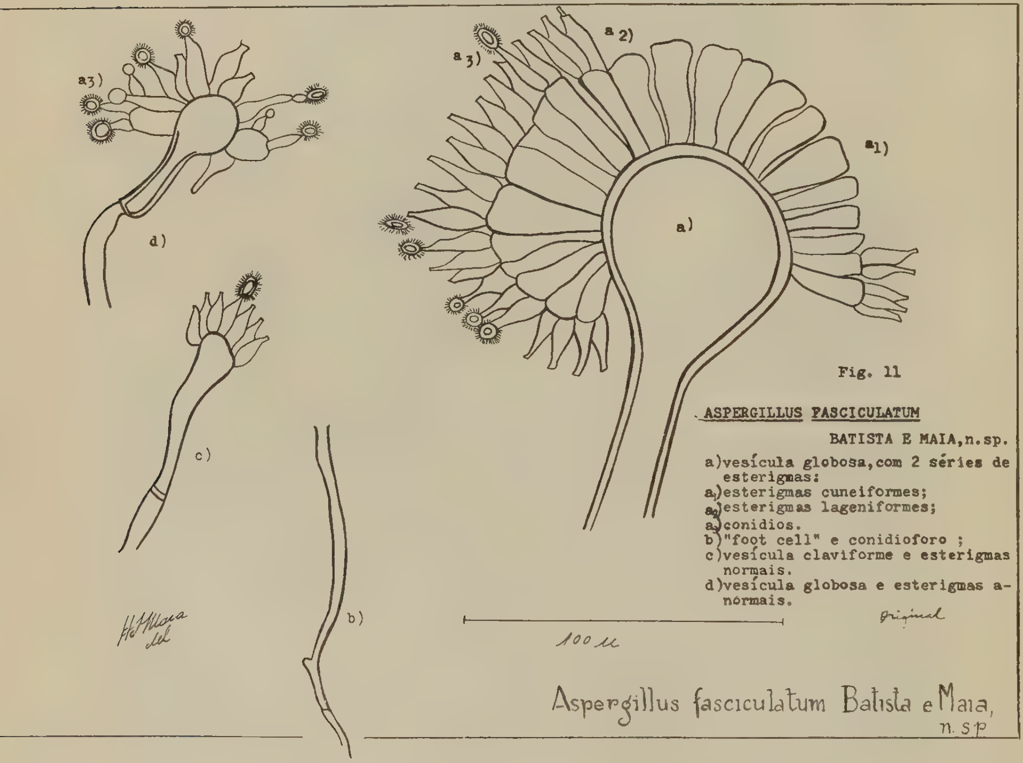
a3)
d)
c)
b)
H. Maia del.
a3)
a2)
a1)
a)
Fig. 11
ASPERGILLUS FASCICULATUM
BATISTA E MAIA, n.sp.
a)vesícula globosa, com 2 séries de esterigmas:
a_1)esterigmas cuneiformes;
a_2)esterigmas lageniformes;
a_3)conidios.
b)"foot cell" e conidioforo ;
c)vesícula claviforme e esterigmas normais.
d)vesícula globosa e esterigmas anormais.
Original
100 µ
Aspergillus fasciculatum Batista e Maia, n. sp

Fig. 11.

uni ou plurisseptados, de 25—35 × 3,5—5,5μ, ou se acham de permeio com esterígmas normais, lageniformes, Fig. 17.

As vesículas proliferantes em pseudo-conidióforos e vesículas secundárias, terciárias e quaternárias, ao contrário, são bastante frequentes, nelas aparecendo pseudo-conidióforos, ou esterígmas modificados, que dão lugar aos entumescimentos vesiculares, Fig. 16.

Os conidios são globosos, subglobosos ou elipsoides, amarelos ou esverdeados, de episporio espesso e espinescente, alguns ,,strains'' exibindo-os com um apículo bem distinto, 3,4—4,5 × 2,8—5μ, isolados ou reunidos em curtas cadeias, de 25—37μ de extensão.

As variações morfológicas das vesículas de que a Fig. 17 nos dá conta, são in-

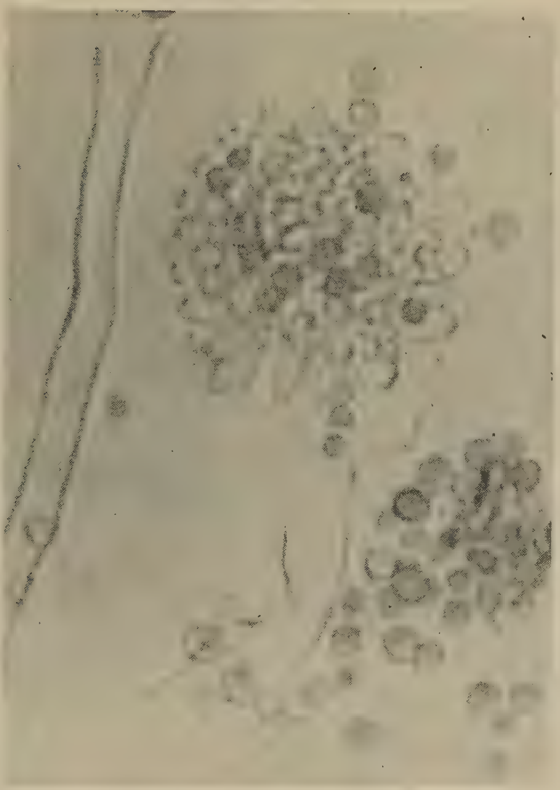

Fig. 12. *Aspergillus fasciculatum* Batista e Maia, n. sp.
Parte de conidioforo e vesícula detalhando a disposição intricada de esterigmas unisseriados. Orig. 400 × ampl. 2 ×.

frequentes em outros meios de cultura e a diferenciação de vesículas secundárias e terciárias é dificil de observada.

Sôbre malte-agar as colonias atingem a 9 cm. de diametro, aos 10—12 dias e à temperatura de 26—28°C, conspicuamente zonadas, planas, de tonalidade verde-amarelada ou verde-cinza, inodoras, sem exsudato, e de reverso branco-sujo, nelas não se desenvolvendo cleistotecios ou esclerocios. Os conidióforos alcançam de

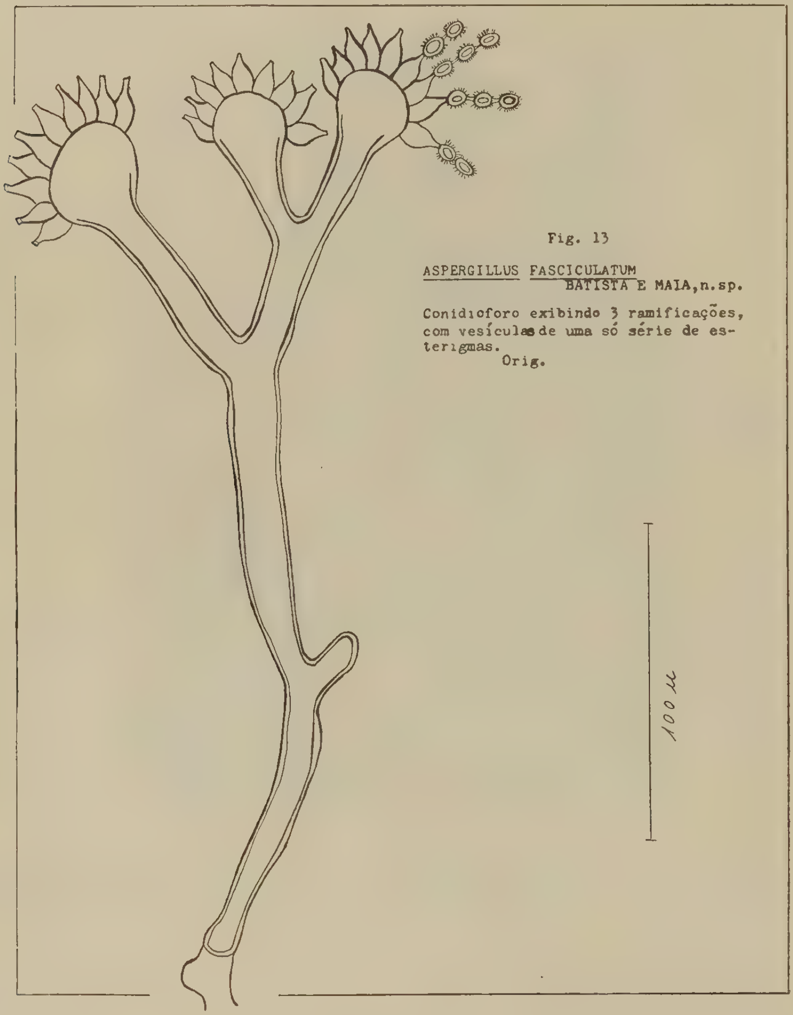

Fig. 13

ASPERGILLUS FASCICULATUM
BATISTA E MAIA, n.sp.

Conidioforo exibindo 3 ramificações, com vesículas de uma só série de esterigmas.
Orig.

Fig. 13

280—500 × 8,4—14μ, permanecendo indivisos ou seja sem ramificações. As vesículas geralmente são de tipo normal, subglobosas com uma única série de esterígmas, e medindo de 7,8—33,7 × 6,7—28μ ou apresentam-se como proliferantes, sua proliferação, entretanto limitada a esterígmas alongados, cujo aspecto até certo ponto se confunde com hifas do micélio. Os esterígmas normais têm o tipo lageniforme, de ápice rombudo, 7,8—11 × 2,2—3,3μ; demais caracteristicas análogas às que foram descritas para Czapek-agar.

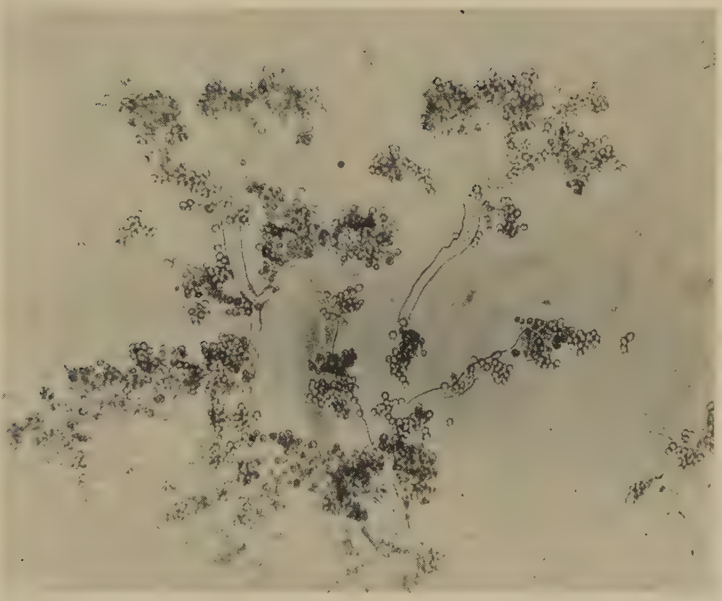

Fig. 14. *Aspergillus fasciculatum* Batista e Maia, n. sp.
Grupos de conidioforos não ramosos e com duas e três ramificações, de aspecto fasciculado. Orig. 200 ×.

Sôbre ,,corn-steep-agar'' as colonias são de crescimento bem menor, chegando somente a 2—4 cms. de diâmetro, aos 10—12 dias, e à temperatura ambiente de 26—28°C; a sua superficie é tambem plana, velutina-flocosa, verde-amarelada, nela não se notando entretanto, zoneamento; não se desenvolvem esclerocios, nem odor, nem exsudato; o reverso é plano, de coloração amarelo-laranja, brilhante. Os conidioforos, vesículas e conidios ocorrem como do tipo normal constituido sôbre Czapek agar.

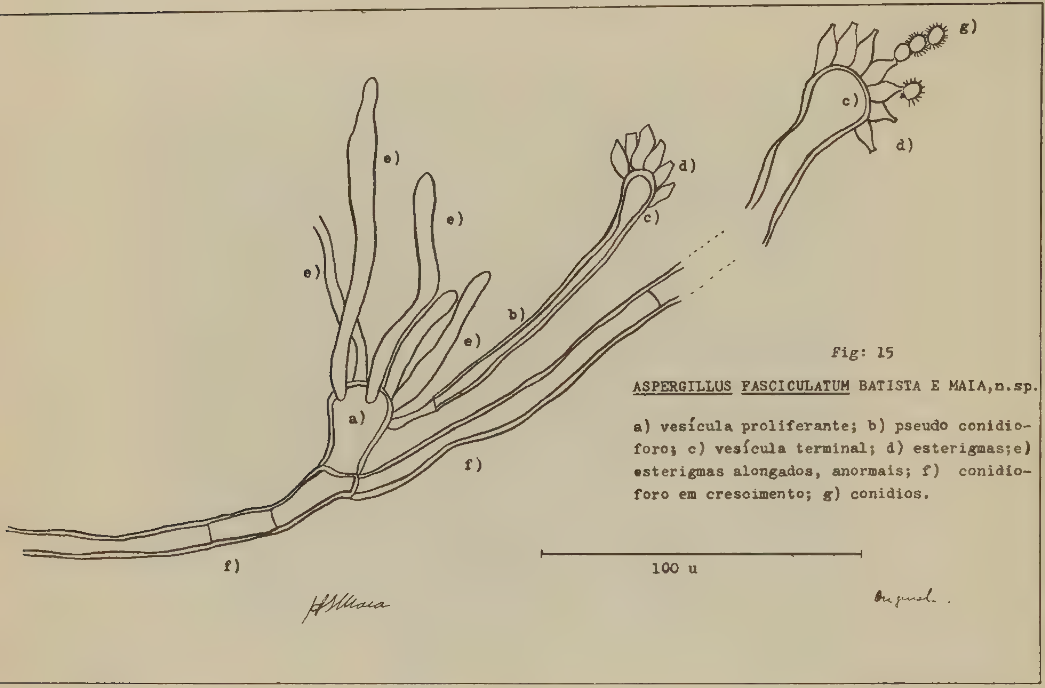

Fig: 15

ASPERGILLUS FASCICULATUM BATISTA E MAIA, n.sp.

a) vesícula proliferante; b) pseudo conidioforo; c) vesícula terminal; d) esterigmas; e) esterigmas alongados, anormais; f) conidioforo em crescimento; g) conidios.

Fig. 15

Em batata-dextrose-agar o desenvolvimento das colonias não ultrapassa de 8 cms. de diâmetro, aos 10—12 dias, e à mesma temperatura ambiente; a superfície das colonias de coloração verde-amarelada, é fortemente zonada, Fig. 18, tambem sem exsudato e sem esclerocios; inodoras; o reverso é branco-sujo ou roseo-amarelado. A frutificação conidial pouco discrepa da de tipo normal, sôbre Czapek-agar.

O fungo ora descrito foi isolado como contaminante de cultura, no Laboratório, em 30.10.52 e tambem como contaminante de *Fusarium sp.*, em 29.9.54. Leg. A. C. Batista. Tipo no 236 isotipo no 101, no Instituto de Micologia, da Universidade do Recife. Enquadra-se no grupo *Aspergillus flavus — oryzae.*

,,Coloniae in agaro Czapekii neutro expandentes, planae, tenues, mycelio vegeto submerso dein aereo-floccoso, pauci zonatae, viride in

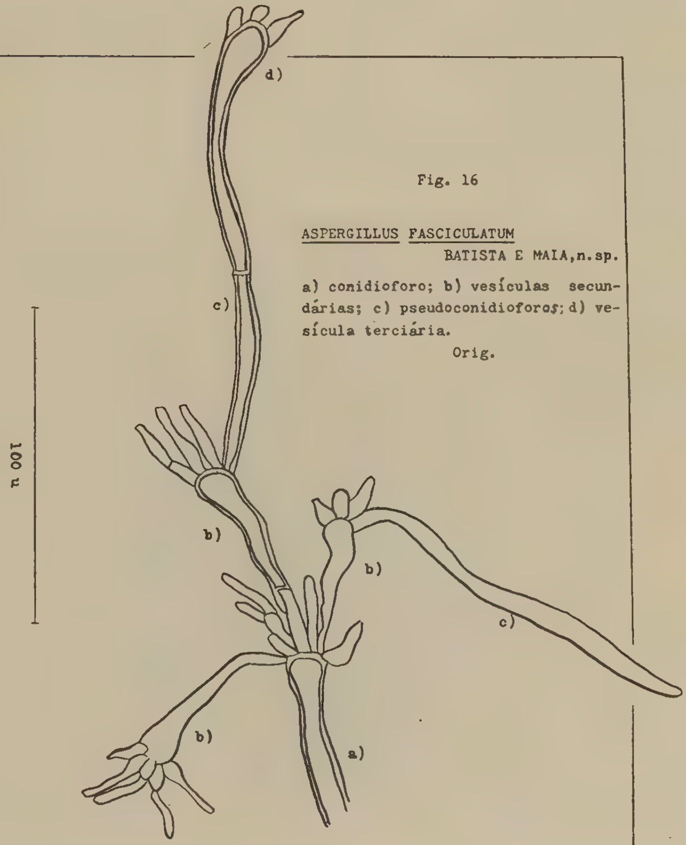

Fig. 16

ASPERGILLUS FASCICULATUM
BATISTA E MAIA, n. sp.

a) conidioforo; b) vesículas secundárias; c) pseudoconidioforos; d) vesícula terciária.

Orig.

HSMaia
del

Fig. 16

centro, flavido in margine, in concentrico — circulos; exsudato odoreque carentibus; reverso roseo flavido in centro, flavido viride in margine. Conidiophoris erectis cum angusta cellula pes, longissimis, 675—1643 × 14—17 *μ vel hiphae ramosae derivatim, septatae vel non, membrana flavida, rugosa, vel formas inaequalitas in apice bifurcatis, vel lateralis ramosarum cum membrana laeves; vesicula normaliae globosae vel conoideae, toto fertilis,* 34—67,5 × 22,5—67,5 *μ, sterigmatibus* 1—2 *seriatim et vesiculae anormaliae truncato-conoideae, hyalinae vel flavidae non totus fertilis,* 16—21 × 7,8—15 *μ, sterigmatibus* 1—2 *seriatim vel prima renidens, cuneiformibus,* 7,8—17 × 5,6—7,8 *μ; secundarius in* 2—3 *verticillis, lageniformis,* 7,8—11 × 5,5—7,8 *μ. In vesiculae proliferantibus, sterigmatibus alicubi elongati usque* 25—35 × 3,5—5 *μ vel toto mutatione in vesiculas secundarias. In vesiculae uni seriatim sterigmatibus lageniformis,* 6,7—10 × 3,3—5,6 *μ. Conidiis globosis, subglobosis vel ellipticis flavidis, echinulatis,* 3,4—4,5 × 2,8—5 *μ, in columnis usque* 25—37 *μ longis. Peritheciis absentis. Sclerotia primum albida, dein nigra, parenchymatica.*

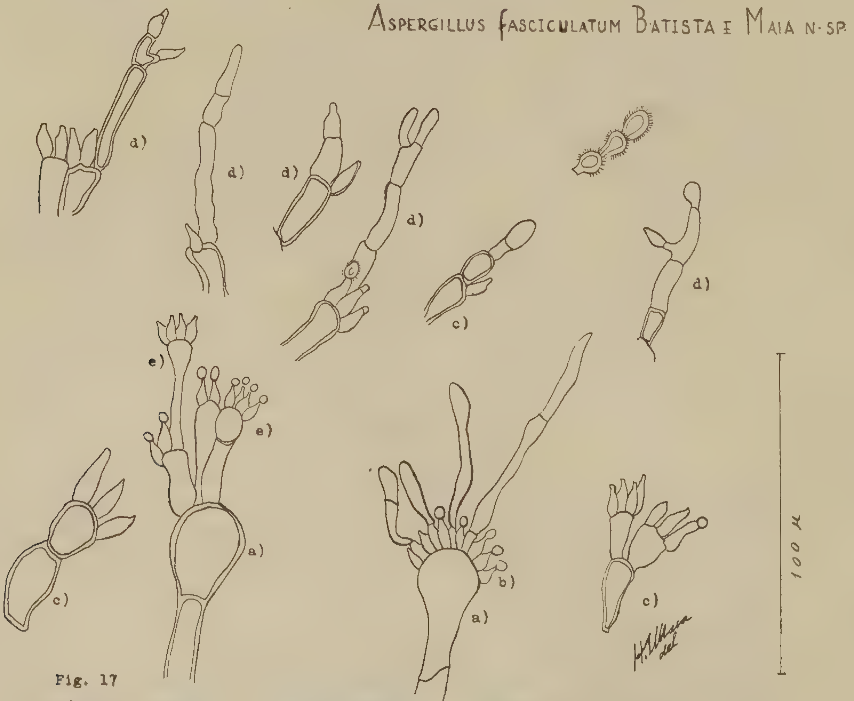

Fig. 17

a) vesículas primárias; b) esterigmas normais; c) esterigmas cuneiformes com duas séries de esterigmas anormais; d) esterigmas alongados, anormais; e) vesículas secundárias.
Orig.

Fig. 17

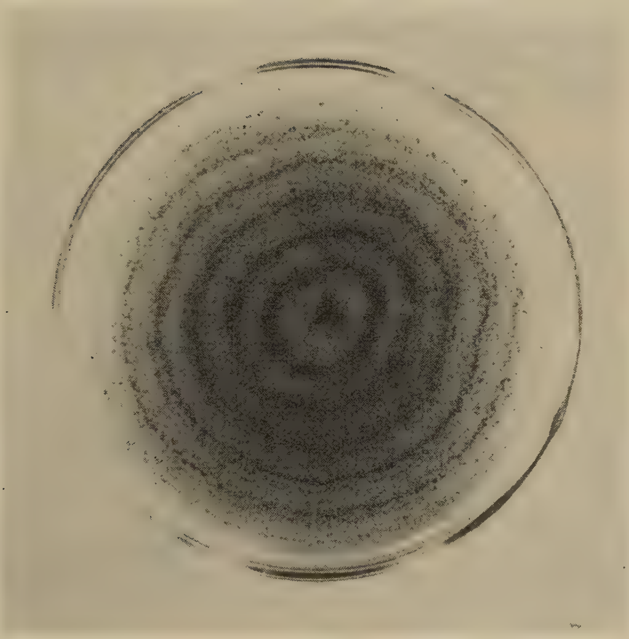

Fig. 18. *Aspergillus fasciculatum* Batista e Maia, n. sp. Colônia, aos 10 dias, em batata dextrose-agar. Orig.

Coloniae in malti-agaro pauci distincte, viride-flavidae vel viride-cinereae, cum reverso albido-sordido; cleistothecia et sclerotia non formantibus. Conidiophoris non ramosis, 280—500 × 8,4—14 μ; *vesiculae globosae, sterigmatibus* 1 *seriatim vel proliferantibus.*

In ,,corn-steep'' agaro coloniae restrictae viride-flavidae, planae, velutino-floccosae, non zonatae; reverso aurantiaco.

In ,,potato-dextrose'' agaro coloniae expandentes viride-flavidae, exsudato et odoreque carentibus, reverso albido-sordido vel roseo-aurantiaco. Conidiophoris et vesiculis similibus cum fructificatione normali in Czapeki-agaro. Cleistotheciis et sclerotiis absentis.

Typus, 236, isotypus 101, Instituto de Micologia, Universidade do Recife. Leg. A. C. Batista, in contaminatione in Laboratorio 30.10.52 et *Fusarium sp.* contaminatione 22.9.54.

Prov. Pernambuci, Brasiliae, Amer. Austr.

Summary

Aspergillus fasciculatum n. sp. has been found as contaminant of Lab. cultures, on 30.10.1952 and again on 29.9.1954. It grows well in Czapek neutral agar, as well as in Malt agar and Potato-dextrose-agar, less intensively in Corn steep agar.

Other media have not been tried so far.

It presents conidiophores smooth or rough, yellowish, septated or not, undivided or branching at the apex, supporting then 2 vesicles or yet with the apex trifurcated or turning into branches, each swelling to form vesicles, side by side, with single head conidiophores.

Abnormal structures in which the sterigma becomes a second conidiophore with a vesicle, were found very common. Normal structures present vesicles with two and one series of sterigmata.

Having its connections within the Flavus-oryzae group, by these outstanding characteristics not reported before, it was considered a new species.

Fig. 1. Autoradiography on silver bromide paper of 5 days old colony of mutant strains of human mycobacteria tuberculosis obtained by action of radioactive cobalt. Exposition time 30 minutes of 12th generation of microbes.

MUTATION OF MYCOBACTERIA TO PROACTINOMYCES BY RADIOACTIVE COBALT

by

Mahmoud Kamal Muftic

(*Mycological Laboratory-Middle Euphrat Hospital; Kuffa; Iraq*)

(1. 9. 1955)

(With plate IV)

Frouin and Guillaumie (1923) estimated that mycobacteria are not schizomycetes but pure fungi. Langeron (1945) believed that tubercle bacilli are with no doubt actinomycetes, and tuberculosis is the most grave and generalised mycosis. There is also an intermediate form between non ramified mycobacteria and abioundantly ramified actinomyces, in genus: Proactinomyces. They are filamentous microbes with traces of ramification. According to Turfitt (1944) and Arnaudi (1949) both mycobacteria and proactinomyces can use cholesterol as the only carbohydrate source.

Mycobacteria being capable of adaptation and mutation with comparative ease, the synthetic and catabolic functions of its strain are susceptible to very wide variations, some of which are direct and fairly rapid adjustments to the environment and others only produced by considerable alteration to the "genes" or their nucleic acids. Nucleoproteids appear to be the self-reproducing units of living matter and an alteration to their nucleic acid results in a permanent alteration to the reproducing units so this affects the properties of the cell's progeny.

Metalnikov, Yaximach and Gelelovitch (1940) succeeded to obtain hereditary mutants in sarcinae by radon. Nadson (1935), Renaud (1943), Guyenot (1942) and Sartory and Meyer (1926) obtained different irreversible mutants by action of radium salts in various fungi. Renon (1913), Bequerel (1913) and Frouin (1920) have examined action of radioactive salts on tubercle bacilli. Their reports proved that low concentration of uranium and thorium salts exert a favourable action on rapidity and intensity of development of surface growth.

Rice, Orr, Reed, and Gardiner (1931) used ultraviolet rays for separation of "S" (smooth) form from "R" (rough) form of tubercle bacilli. Form "R" is more sensitive to ultraviolet and rapidly perishes and form "S", so obtained, is less virulent than usual.

In our essays on mutation of mycobacteria we used "S" form of mycobacteria tuberculosis hominis, cultivated in Dubos Tween-Albumin liquid media with soluble salt of radioactive isotope of cobalt (Co^{60}) which has a radiation energy in gamma-rays of 1.3 MEV. We chose cobalt isotope only because it has soft gamma radiation of long life, and "S" form of tubercle bacilli, because it is less sensitive to the lethal effect of short rays.

Experimental

A strain of tubercle bacilli form "S", obtained from pulmonary lesion at necropsy, was studied in about ten reinoculations in Loewenstein eggs-medium and by inoculation in guinea pigs.

We prepared three series of liquid Dubos Tween-Albumin medium.

A – Medium with different concentration of radioactive cobalt chloride.
B – Medium with different concentration of non-active cobalt chloride.
C – Medium serving as control without cobalt salts.

After incubation of three weeks we inoculated all media with 0.1 mg of moist weight of fresh bacillary culture from Loewenstein medium. Cultivation was carried on for four weeks and the colonies were reinoculated on Loewenstein medium.

In media "A" with 100—200 μCu per ml we obtained a strain, different from the mother strains, so that we can estimate it as a mutative type, with hereditary reproduction of all characteristics. We designed this type as Mycobacterium tuberculosis hominis (isotope Co^{60}, chromogene, virulent) H_{60} Ch_v.

The new mutant strain is polydynamic and dysgonic. Its colonies have a thick, creamy, rough surface growth, pigmented oker-purple and occasionaly okerviolet. It rises on walls of bottles, covers surface completly in a very short time. These polydynamic properties of the new strain are rare in pathogenic mycobacteria and are comparable only to the avian type. After only five days of incubation on Loewenstein media all surface was covered. The quantity of growth was about sixty times greater than that of mother strains obtained from culture "B" and "C" after 42 days of incubation.

Microscopically the mutant looked like coenotic rarely ramified mycelium of very weak acid-fastness and with a fine iodophylic granulation. Also a typical Proactinomyces.

It consumed: glucose, maltose, trehalose, inosit, glycerol, cholesterol, paraffine and naphta-petrol. It reduced methylene blue, and decomposed: amygdalin, salicin and arbutin.

The guinea pigs inoculated intraperitoneal by H_{60} Ch_v die in the third week. At the end of the second week we can isolate the germs from blood. Necropsy showed many mycetomes in lymphatic glands and lung. A week after inoculation the animals were sensitive to tuberculin. The rabbits are of the same sensitivity to inoculation. They perished in the fourth week with formation of mycetome in mesenteric glands. The fowels are refractery to the infection.

Comparative pathogenetic study

30 guinea pigs were intraperitoneally inoculated by 0.1 mg of original strains of tubercle bacilli. They died in between 8—14 weeks. Necropsy showed general granular tuberculosis in abdominal viscera, thoracic ganglions with engorgement and caseification of retrosternal glands and formation of tuberculous nodules on the omentum. The rabbits were resistent to the infection.

Another group of 30 guinea pigs, inoculated by strains developed in presence of non-active cobalt (0.2 mg per ml), has shown the same pathological characters as above. Survival time was the same. In both groups of animals the spleen and liver were enlarged.

The third group of 30 guinea pigs was inoculated by mutant strain. They succumbed in the third week. Animals showed tumefaction of visceral and mesenteric glands, formation of mycetomes in neck glands, omentum and mucous membranes. Lung, spleen and liver were only slightly enlarged and without tubercle formation. In table No I and II the quantitative amount of infection found at necropsy time in some animals is represented.

Discussion

It is well known that the chromogen form of tubercle bacilli by their antigen structure is nearer to the smooth form than to the rough. At the same time they are less virulent than S form, which is less virulent than R form. It is for this reason more characteristic that the new type, inspite of its chromogene characters, is more virulent than any other strain of mycobacteria including H_{37} R_V.

Photodynamic action of soft gamma-rays of Co^{60} on mycobacteria can be interpreted or by Roussel effect (FRANCIS, MADINAVEITIA, MACTURK and SNOW, 1949) (LACASSAGNE, LATARJET, 1934) due to the presence either photodynamic dyes as vitamin K_2 (FIESER, CAMPBELL and FRY, 1939) (PARSHIN 1946) a naphtoquinon pigment in membrane of bacilli or as absorption effect on nucleoprotides or nucleic acids, the absorption coefficient of which is very strong in short ultraviolet and soft X-ray (VLES, 1946).

As results of radiation shocks SUBRAMANIAM, RANGANATHAN and KRISHNA (1948) observed gene mutation, PREMA BAI (1947) chromosomal translocation, and SUBRAMANIAM, RANGANATHAN (1948b) tetraploidy. It is doubtless that in our experiments the reducing effect on "genes" of mutant stabilised the mycobacteria in the parent form of Proactinomyces. The process during our observation was irreversible. The radiation power of mutant strain is preserved during many generations. Picture 1 shows an autoradiography of the 12th generation culture $H_{60}Ch_V$ on Loewenstein medium. The amount of radiation diminished by time. There is no sign of mutation in other cultures treated by the same concentration of non radioactive cobalt. Thus, it is nòt the polydynamic effect of cobalt as iron-like metal which is responsible for the mutation. Mutation of Mycobacteria to Proactinomyces by radioactive cobalt.

TABLE I

No of animal	Weight pre-infect. Gm	Weight post-infect. Gm	Spleen Gm	Liver Gm	Survival w.
7	400	370	3,5	10,5	3 weeks
9	360	400	3,9	18	2
10	420	400	4	23	3
15	500	475	4	11	3

Some of guinea pigs inoculated intraperitoneally by 0.1 mg of fresh culture of $H_{60}Ch_V$ from Loewenstein medium.

TABLE II

No of anim.	Weight pre-infect. Gm	Weight post-infect. Gm	Spleen Gm	Liver Gm	Survival w.
3 "B"	450	375	9	31	9 weeks
6 "B"	500	360	7,5	46	8
7 "C"	500	510	4	30	11
28 "C"	360	350	6	31	14
29 "B"	420	400	11	26	13

Some of guinea pigs inoculated by:
Group "B" (cobalt non radioactive culture of tubercle bacilli 0.1 mg intraperitoneally).
Group "C" (control culture of tubercle bacilli without cobalt 0.1 mg intraperitoneally).

The new mutant strain of tubercle bacilli is a fairly convincing proof that mycobacteria are really fungi, and also tuberculosis a mycosis.

But as LANGERON (1945) writes, tuberculosis is not studied from this point of view. It became a social disease, that means a disease which let nearly the same number of individuals live as it kills. This is the biological equilibrium and that satisfies the law of moderation.

Summary

A smooth strain of human tubercle bacilli exposed in liquid medium to the gamma irradiation of radioactive cobalt was transmu-

tated in a new type ressembling genus Proactinomyces. Morphologically it showed a rarely ramified mycelium of very weak acid-fastness, and high virulence for guinea pigs and rabbits. It was designated as $H_{60}Ch_V$.

Resumé

Une souche de bacilles tuberculeux humains le type "S" (glabre), cultivée dans un milieu liquide, était exposée au rayonnement gamma du cobalt radioactif, provoquant une transmutation dans un type nouvel ressemblant au genre proactinomyces. Morphologiquement il se représent comme un mycel microsiphoné, avec des ramifications en trace et d'une acido-resistence très faible. Il est très virulent pour les cobayes et les lapins. L'abréviation pour cette souche est: H_{60} Ch_V.

Acknowledge

The supply of radioactive cobalt salts (Co^{60}) was made by PHILIPS-ROXANE, Pharmaceut.-Chemisch. Industr. "DUPHAR", Amsterdam, the Netherlands, to whom we due our best thanks.

Kuffa-Iraq, End of July 1955.

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ALGUNS *AGARICACEAE* SAPROFITOS DE PERNAMBUCO

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(15.1.1955)

(Com 6 figuras)

Hygrophorus pernambucensis Batista e Vital.

Pileu de 6—10mms de diâmetro e 2mms de altura, convexo a princípio tornando-se depois convexo plano, com ,,umbo'' saliente, de margens encurvadas, desprovido de marcas, glabro, porém estriado, de côr marron-laranja, persistente, algumas vezes entretanto o disco central apresentando-se amarelo; contexto fino, ceráceo, frágil, amarelado, sem sabor e inodoro; trama do piléu homogenea, sem diferenciação de película.

Lâminas subdistantes, subdecurrentes, (18—20, alcançando o estipe), marginadas ou de bordos ligeiramente crenulados, simples, de tonalidade amarela, persistentes.

Basidios cilindraceo-clavados, quadrisporos, 25—47,5 × 10—12,5μ, com esterígmas cônicos, de 5—7,5 × 2,5—3,5μ.

Queilocistidios numerosos, subcilindricos ou elipsoides, de ápice agudo, 20—47,5 × 9—15μ.

Leptocistidios clavados, de membrana fina, 20—34 × 5—10μ.

Pleurocistidios ausentes.

Trama das lâminas, de hifas intrincadas ou subparalelas, de coloração amarelo-pálido.

Basidiosporos elipsoides ou lacrimiformes, hialinos, amarelados em iodo, de epispório liso, 11—15 × 5—7,5μ.

Haste central, fortemente aderente ao piléu 3—3,5 cms. de altura e 1—2mms de diâmetro, da mesma côr do piléu, igual, delicada, tubular, com rizoides.

Sôbre serrapilheira, nas matas do Jardim Zoo-Botânico do Recife — Dois Irmãos — Leg. Osvaldo Soares, 14.11.54. Tipo, no 1116, no Instituto de Micologia, da Universidade do Recife. Dets. A. Chaves Batista e A. Fernandes Vital.

,,*Pileus* 6—10 *mm latus et* 2 *mm alt, convexus demum convexus-planus, umbonatus, striatus, brunneo- aurantiacus; lamellae subdistantes, subdecurrentes, ripa crenulatae, luteae; basidia cylindraceo-clavata, tetraspora,* 25—47,5 × 10—12,5 μ, *sterigmatibus conicis,* 5—7,5 × 2,5—3,5 μ. *Cheylocystidiis subcylindraceis vel ellipsoideis,* 20—47,5 × 9—15 μ. *Leptocystidiis clavatis,* 20—34 × 5—10 μ. *Pleurocystidiis absentis. Sporae ellipsoideae vel lachrymiformiae, hyalinae, leve,* 11—15 × 5—7,5 μ. *Stipe* 3—3,5 *cm longis,* 1—2 *mm crassis, concoloribus, aequalis.''*

In silva Jardim Zoo-Botanico — Dois Irmãos, Recife, Leg. Osvaldo Soares da Silva, 14.11.54. Typus, 1116, Instituto de Micologia, Universidade do Recife, Prov. Pernambuci, Brasiliae, Amer. Austr. — Dets. A. Chaves Batista et A. Fernandes Vital.

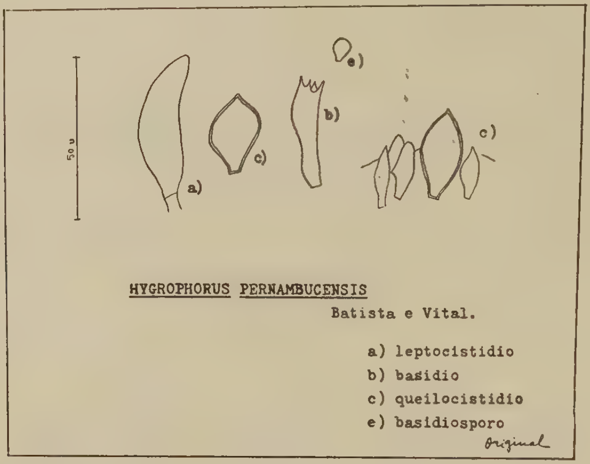


Fig. 1

Lepiota minuta Batista.

Piléu convexo, depois expandido, suavemente umbonado 0,5 cms. de diâmetro, isolado, superfície sêca, estriado-granulada, ferruginea, porém de mamilo mais escuro, bordos inteiros; contexto de 1 mm de espessura, ocre, inodoro. Lâminas largas, ventricosas, recurvadas ou sinuosas, amareladas; inalteráveis, de bordo livre diminutamente franjado; basidios clavados, quadrisporos, 15—20 × 5—7,5 μ; cistidiolos elipsoides, hialinos, 15—17,5 × 6—7,5 μ. Esporos elipticos, lisos, hialinos, com um apiculo distinto, 5—7,5 × 2,5—3,5 μ. Haste igual, lisa, glabra, ferruginea, 4,5—5 cm de altura e 0,8—1 cm de diâmetro; anel superior subcortiniforme, fixo, persistente, branco.

Sôbre serrapilheira, na mata.

Tipo, no 982, Instituto de Micologia, Universidade do Recife — Pernambuco — Brasil. Leg. Osvaldo Soares da Silva. 25.9.54 — Jardim Zoo-Botanico — Dois Irmãos, Recife.

„*Pileo convexo-expanso, sub umbonato,* 0,5 *cm lato, striato-granulato, ferrugineo; lamellis ventricosis, ochraceis fimbriatis; basidiis clavatis,* 4-*sporis,* 15—20 × 5—7,5 *μ; cystidiolis ellipsoideis,* 15—17,5 × 6—7,5 *μ. Sporae ellipsoideae, laevibus, hyalinae, apiculatae,* 5—7,5 × 2,5—3,5 *μ. Stipe aequal, concoloribus,* 4,5—5 × 0,8—1 *cm; annulo supero subcortiniformis, albo*".

In silva folia dejecta.

Typus, 982, Instituto de Micologia — Universidade do Recife — Leg. Osvaldo Soares da Silva, 25.9.54. Jardim Zoo-Botanico — Dois Irmãos — Recife. Prov. Pernambuci, Brasiliae Amer. Austr.

Marasmius coccineatus Batista.

Piléu glabro hemisférico, depois convexo, com a porção central deprimida na fórma de disco, tornando-se assim subumbilicado, com 1—1,5 mms de diâmetro; margens sulcadas e bordos denteados; coloração escarlate intenso, a qual se conserva inalterada nos especimens sêcos, ou se transforma em amarelo laranja, no líquido de Hessler; contexto de côr laranja, escasso, inodoro.

Lâminas vermelhas, livres, dístantes, ventricosas, não amiloides, de bordo inteiro.

Pleurocistidios clavados, hialinos, 13—56 × 6—10 µ.

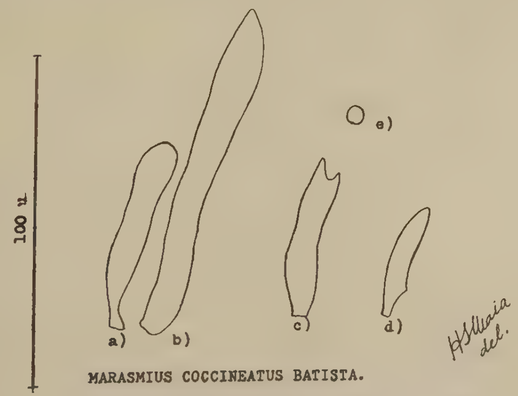
Cistidiolos cilindraceos, 11—16 × 4—7,5 µ. Queilocistidios ausentes. Basídios elipsoides, cilindro-truncados, de base mais larga, quadrisporos e bisporos, 10—12 × 7,5—10 µ, com esterigmas filiformes, até 4 µ de extensão.

Basidiosporos globosos, hialinos, não amiloides, 2,5—5 µ de diâmetro. Estipe também escarlate, como o piléu, de ápice subfiliforme, atenuado, corneo ou cartilagineo, glabro, flexuoso, 7—15 mms de altura e 0,2—0,5 mms de diâmetro.

Sôbre fôlhas mortas e sementes de páu ferro — *Cesalpinia ferrea Mart* — Jardim Zoo-Botânico-Dois Irmãos-Recife — Leg. Osvaldo Soares da Silva. — 10/IX/54. Tipo, no 938, no Instituto de Micologia da Universidade do Recife.

„Pileo sanguinolento semiglobato, dein plano-convexo, sub-depresso vel subumbilicato, glabro, 1—1,5 *mm, margine sulcato; lamellis concoloribus, liberis, distantibus, ventricosis inamyloideis; carne exigua, aurantiaca inodora; pleurocystidiis clavatis, hyalinis,* 13—56 × 6—10 µ. *Cystidioliis cylindraceis,* 11—16 × 4—7,5 µ. *Cheilocystidiis nullis; basidiis ellipsoideis vel cylindro-truncatis,* 4 *vel* 2*-spori,* 10—12 × 7,5—10 µ; *sterigmatis filiformibususque* 4 µ *longis. Sporis globosis,* 2,5—5 µ *diam, hyalinis, non amyloideis. Stipite sanguinolentus, concoloribus cum pileu, ad apicem subattenuato, corneis vel cartilagineis, glabro, flexuoso,* 7—15 *mm alt et* 0,2—0,5 *mm diam."*

In folia mortua *Cesalpinia ferrea* Mart-Jardim Zoo-Botânico-



MARASMIUS COCCINEATUS BATISTA.

a-b) pleurocistidios; c) basidio; d) cistidiolo
e) basidiosporo.

Orig.

Fig. 2

Dois Irmãos-Leg. Osvaldo Soares da Silva, 10/IX/54. Typus, no 938, Instituto de Micologia, Universidade do Recife-Prov. Pernambuci, Brasiliae-Amer. Austr.

Pseudocoprinus brasilianus Batista n. sp.

Piléu 4—6 mms. de altura e 15—20 mms. de diâmetro, cônico a principio, depois, convexo-hemisférico, glabro, pliçado-estriado, radialmente, de margens involutas à maturidade, cinereo, por fim argilaceo claro, membranoso-cartilagineo; *trama* delicada, membranosa inodora, creme; *lâminas* adnatas, iguais, muito juntas, uma ou outra lamelula presente, cinereas quando jovens, tornando-se negras com o aparecimento dos espóros, coloração essa que se mantém persistente; — *haste* 2,5—3,5 cms. de comprimento, 1—2 mm de diâmetro, igual, branca, depois argilacea, cartilaginea, fortemente aderente ao piléu, devido a uma profunda implantação. *Esporos* negros em depósito, marron escuro em potassa a 10 %, subtriangulares ou subelipsoides, 8—10 × 4,5—8μ, poro indistinto; *basidios* dimorficos, 2—4 esporos, predominantemente bisporos, 23—30 × 8—16 μ, clavados, com *esterígmas* de 3,5—4,5 × 2—2,5 μ; *cistidiolos* coprinoides; *pleurocistidios* frequentes, 57—125 × 12—19 μ; *queilocistidios* pedicelado-vesiculosos, raros, 56—90 × 22—25 μ; hialinos em potassa; leptocistidios clavados, 33—45 × 10—13 μ, raríssimos.

Gregario, sôbre solo arenoso, na mata. Tipo, 900 no Instituto de Micologia, Universidade do Recife, Jardim Zoo-Botânico Dois Irmãos — Recife. Leg. Osvaldo Soares da Silva, 20.8.54.

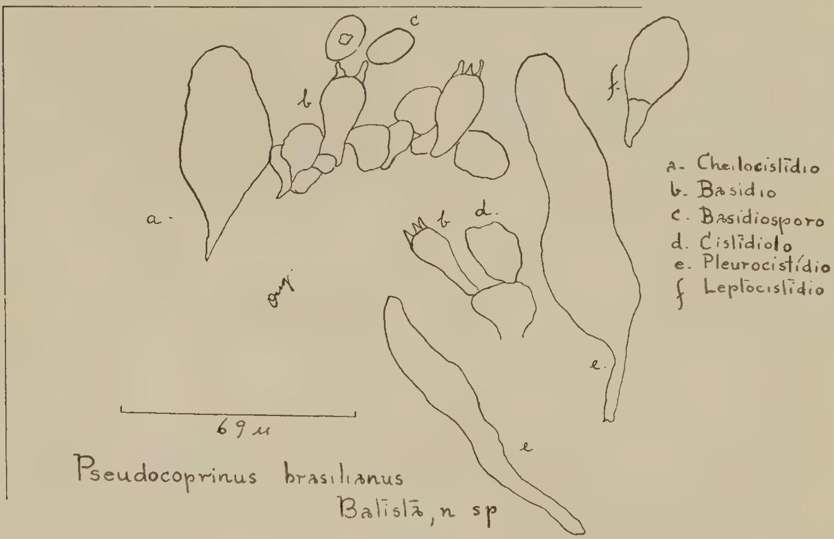


Fig. 3

„Pileus 4—6 mms altus, 15—20 mms. latus, conicus demum convex-hemisphaericus, glaber, plicatostriatus, cinereus demum argillaceus, membranoso-cartilagineus; pileus trama membranosa, inodora; lamellae adnatae, aequalae, angustae, leucorinae demum nigrae; stipes 2,5—3,5 cm. long, 1—2 mm diam, aequalis, argillaceus ad maturitatem, cartilagineous; sporae nigrae ellipsoideae vel subtriangulariter,

8—10 × 4,5—8 μ; *basidia dimorphica, bi vel quadrispora,* 23—30 × 8—16 μ; *sterigmata* 3,5—4,5 × 2—2,5 μ, *cystidiola coprinoides; pleurocystidia* 57—125 × 12—19 μ; *cheilocystidia pedicellato-vesiculosa, rara,* 56—90 × 22—25 μ *leptocystidia clavata,* 33—45 × 10—13 μ".

Gragaria in terra arenosa. Typus, 900, Instituto de Micologia, Universidade do Recife — Leg. Osvaldo Soares da Silva, — 20.8.54 — Jardim Zoo-Botanico — Dois Irmãos, Recife. Prov. Pernambuci Brasiliae, Amer. Austr.

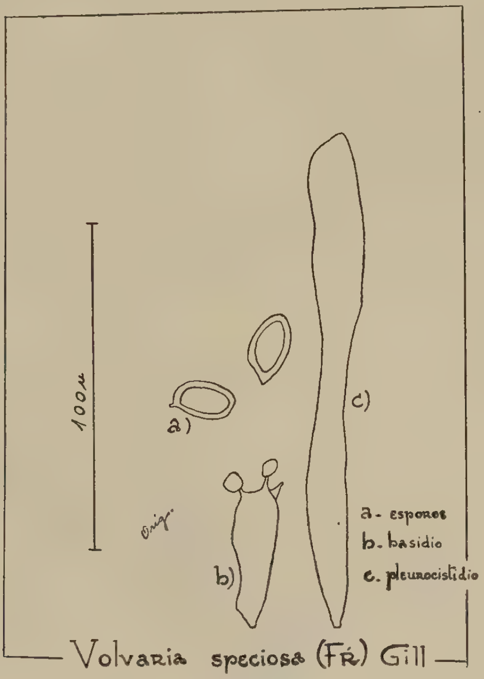


Fig. 4

Volvaria speciosa (Fr.) Gill.

Piléu campanulado — expandido, conservando-se porém umbonado, 5—8 cms. de diâmetro, viscoso, glabro, de tonalidade branco-rosea; epiderme não aderente; bordos finos, franjados; contexto branco, compacto; frágil, mas fortemente aderido à haste; translúcido, sob incidência de luz direta; descora-se rapidamente, quando imerso em alcool; odôr agradável.

Lâminas inicialmente brancas, depois roseas e por fim de côr marron, finas, largas e livres ou adnexas, com bordo liso, de ponta obtusa, pouco aderentes ao piléu.

Haste firme, central, 8—10 cms. de altura e 1—2 cms. de diâmetro, branca, atenuada, subbulbosa, vilosa, desprovida de marcas, de consistência fibrosa, ôca, sem anel.

Volva branca, vilosa, lobada, até 1,5 cms. de altura.

Basidios clavados, 27,5—47,5 × 10—15 μ, com esterígmas curtíssimos e espessos.

Cistidios lanceolados (pleurocistidios) 62,5—162,5 × 12,5—20 μ.

Esporos vermelho-ferrugíneos em depósito, amarelo-esverdeados ao microscópio, sob hidrato de cloral; elipsoides, de epispório espesso, com apículo distinto, 9—16 × 7,5—9 μ.

Sôbre solo humoso, rico em estêrco de curral.

Especimen No. 861, Instituto de Micologia, Universidade do Recife.

Leg.: Hellis Lacerda. Beberibe, Recife — 27.8.1954.

Nota: — Consultar P. A. SACCARDO — Em Flora Italica — Cryptogama — Hymeniales — pag. 515, 1915. Tambem WAKEFIELD E. M. and R. W. G. DENNIS — Em Common British Fungi, pag. 133, 1951.

Volvaria oswaldoi n. sp. Agaricaceae-Rhodosporae.

Piléu carnoso, globoide a principio, depois campanulado-expandido, 6—12 cms de diâmetro, conservando-se porém, sub umbonado, de superfície sub glutinosa quando jovem, fibrilo-sedoso-escamosa, no estado adulto, de margens estriadas, branco, sarapintado de marron 682, com exceção da região apical onde quasi desaparece o branco sob o predomínio da tonalidade marron referida; a região umbonada fendilha-se, em sulcos irregulares e profundos, à maturidade, interessando também nêsse fendilhamento à porção implantada da haste; trama flocosa, flácida, branca, inalterável por exposição ao ar, em 24 horas, muito fina para os bordos do piléu, chegando, entretanto, a 1,2—1,5 cms. de espessura, na área central; odôr e sabor indistintos.

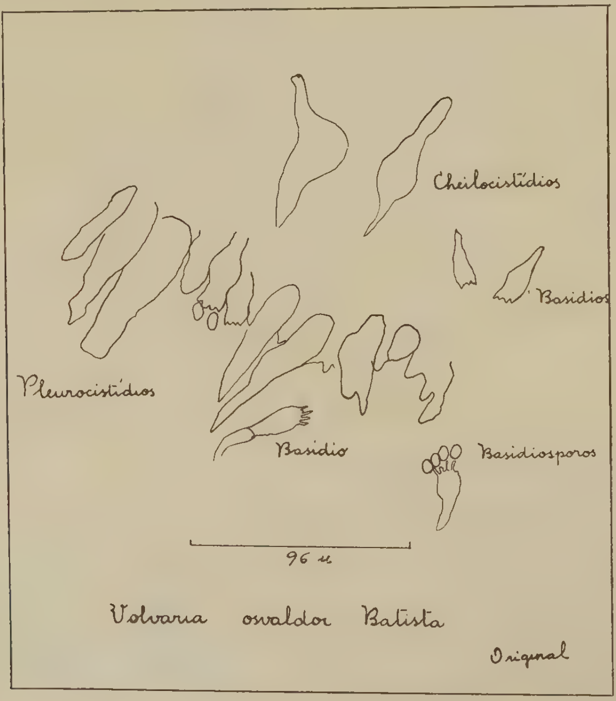
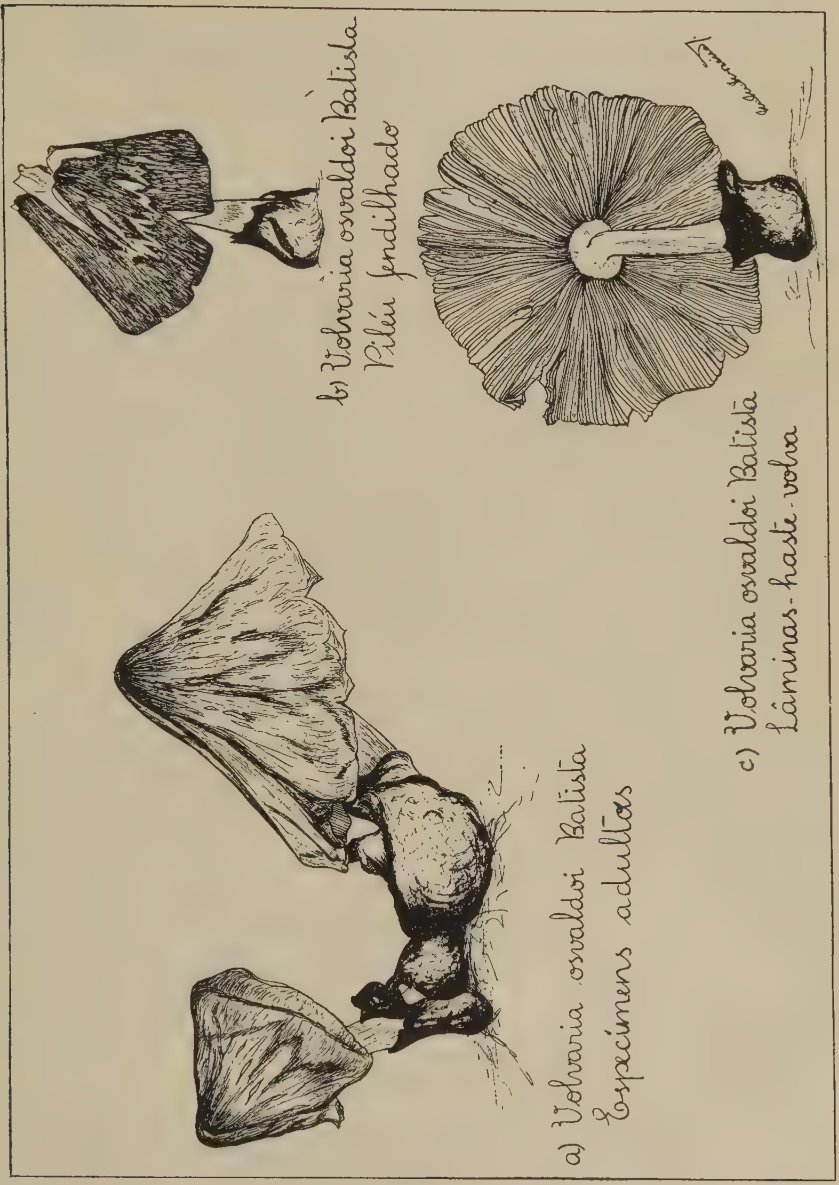


Fig. 5



a) Volvaria osvaldoi Batista
Especimens adultos

b) Volvaria osvaldoi Batista
Piléu fendilhado

c) Volvaria osvaldoi Batista
Lâminas-haste-volva

Fig. 6

Lâminas livres, deixando um colar branco de 0,3—1,2 cms. de diâmetro em torno da haste, de côr laranja 200, inicialmente, e depois roseo-marron 710, de bordos ligeiramente erosados.

Basidios claviformes, 25—30 × 7—10 μ, estreitados na base, quadrisporos; esterígmas triangulares, de 2,5—3 × 1 μ.

Queilocistídios em quantidade apreciável, clavado-setiformes ou fusoide-ventricosos, de ápice agudo ou filiforme, 67,5—75 × 20—25 μ.

Pleurocistídios pouco frequentes, incolores sob potassa, de paredes delicadas, prismáticos ou cônicos, de ápice obtuso, 37,5—62,5 × 7,5—10 μ.

Grampos de conexão não observados.

Esporos marcantemente elipsoides, alguns globosos 5—10 × 5—7,5 μ, de episporio liso, leves, regulares, apiculados, vermelho 133 ao microscopio e vermelho 102 em depósito; ,,spore print'' muito bem formado em 4 horas, que aliás é tempo excessivo para o delineamento radiante das lâminas. Especimens adultos, postos em solução alcool-água 50 %, não descoram as lâminas, formando porém uma suspensão avermelhada com os esporos, a qual, em repouso, só se torna límpida depois de 24 horas.

O volume total de esporos produzidos em 24 horas é estimado em 2.000.000.000.

Haste sólida, central, profundamente implantada no piléu de onde não se destaca sem laceração, branca, fibrilosa, 5—9 cms de extensão, dilatada na base, glabra, sericea, sem marcas, direita ou encurvada, inserida na taça volvar; diâmetro basal 2—2,5 cms e diâmetro apical 0,8—1,2 cms.

Volva frouxa, membranosa fina, lobada na margem superior, nívea de começo e e por fim de côr laranja 200, vaginante, 5—6 × 3—3,5 cms.

Reações químicas: KOH, FENOL, NH_3, NH_4OH, $FeSO_4$ e Anilina negativas; H_2SO_4, na superfície do piléu coloração rósea; na trama tonalidade vermelha; *HCl* na superfície do piléu e na trama, coloração rósea.

Fototropismo positivo; entomotactismo negativo.

Habitat — Sôbre detritos de algodão, em solo úmido.

Legador: José Francisco da Silva, 12.8.1954. Beberibe, Recife — Tipo 872, Instituto de Micologia, Universidade do Recife.

Designação especìfica — Em honra ao Prof. Oswaldo Gonçalves de Lima, microbiologista brasileiro.

,,*Piléu carnosulo, globoide dein campanulato expanso,* 6—12 *cms. lato, subumbonato, subglutinoso ab initio, dein fibrillos sericeo squamoso, albo-brunneo* 682, *ápice brunneo* 682 *immaculato, in centro lacerato ad maturitatem, margine striato; trama floccosa candida,* 1,2—1,5 *cms. crassa in area centralia; lamellis liberis, roseo-brunneis* 710, *marginis erosatis; basidiis clavatis,* 25—30 × 7—10 μ; *sterigmatibus triangularis,* 2,5—3 × 1 μ; *cheylocystidiis clavato-setiformis vel fusoide-ventricosis,* 67,5—75 × 20—25 μ; *pleurocystidiis pauci numerosis, prismaticis vel conicis, ápice obtuso,* 37,5—62,5 × 7,5—10 μ; *sporis ellipsoideis vel globosis,* 5—10 × 5—7,5 μ *levibus, regularibus, apiculatis, rubentibus* 102 *in deposit; stipite solido glabro, sericeo, attenuato, recto vel incurvato,* 5—9 *cms. longo, bulboso; volva laxa membranosa, lobata in margine superiore, nivea dein aurantiaca* 200, *vaginantia,* 5—6 × 3,5 *cms.''*

In lana lignea dejecta.

Leg.: José Francisco da Silva. 12.8.1954 — Beberibe Recife. Typus, 872, Instituto de Micologia Universidade do Recife — Prov. Pernambuci, Brasiliae, Amer. Austr.

In honoris Prof. Oswaldo Gonçalves de Lima.

Nota: — The color nomenclature is based in ,,Code Universal des Couleurs'' by E. Séguy — ed 1936 Paul Lechevalier, 12 Rue de Tournon Paris.

IN VITRO STUDY OF ANTIFUNGAL ACTIVITY OF PENTAMIDINE AND STILBAMIDINE

H. S. Andleigh

(*From the Department of Pathology and Bacteriology, Sawai Man Singh Medical College, Jaipur.*)

(12.7.1955)

Elson (1945) made the observation that propamidine had antifungal activity. The discovery by Elson of the fungistatic activity of propamidine (p.p. Trimethylendibenzamidine) against a number of pathogenic fungi led to further confirmatory investigations of other aromatic diamidines by Bocobo et al (1953) by Christison & Conant (1953) and by other investigators. The other aromatic Diamidines which were tried were pentamidine (p.p. pentamethylenedibenzamidine) and stilbamidine (p.p. stilbene diacarboxamidine). Singular success has been obtained with the use of stilbamidine in the therapy of North American Blastomycosis. As such it was considered worthwhile to study the antifungal activity of pentamidine and stilbamidine against the organisms isolated from cases of Maduromycosis (Black granule type) in India.

Material and Methods

Antifungal activity was measured by the serial dilutions in liquid media method similar to that described by Bocobo, et al. (1953). Brain heart infusion liquid medium with 20 units of penicillin and 30 units of streptomycin was used for the growth of organisms isolated from cases of Maduromycosis. The cultures isolated from such cases were all of *Madurella mycetomi*. Five of these cultures, after their primary isolation, were tested for their sensitivity against both pentamidine and stilbamidine and the test was subsequently repeated several times for each strain.

Brain heart infusion liquid medium was prepared as follows: 37 gms. Difco's Dehydrated Brain Heart Infusion, were added to 1000 ml. of distilled water in a two litre flask.

The material was dissolved in the autoclave in steam and, when dissolved, it was sterilised at 15 lbs. pressure for 20 minutes. After the media had cooled to 35° C., penicillin and streptomycin was added in the proportion of 20 units of penicillin and 30 units of streptomycin per ml. of the medium. To this pentamidine and stilbamidine were added directly, setting up serial dilutions of the compounds in concentrations of 0.3 mg., 0.2 mg., 0.1 mg. and 0.01 mg. per ml. of the medium. No solvent was needed because the chemicals dissolved directly in the medium. While pentamidine made a perfectly clear and transparent medium, the medium containing stilbamidine was slightly opalescent.

Growth was observed at 37° C. and the end point was determined at the lowest concentration of the compound where complete inhibition of the growth occurred.

Results

The results of the experiments are given below in Tables I and II

TABLE I. *Pentamidine concentrations*

Culture Numbers.	0.3 mg. per ml.	0.2 mg. per ml.	0.1 mg. per ml.	0.01 mg. per ml.
1.	—	—	—	+
2.	—	—	±	+
3.	—	—	—	+
4.	—	—	—	+
5.	—	—	—	+

+ = appreciable growth.

TABLE II. *Stilbamidine concentrations*

Culture Numbers.	0.3 mg. per ml.	0.2 mg. per ml.	0.1 mg. per ml.	0.01 mg. per ml.
1.	—	—	+	+
2.	—	—	+	+
3.	—	+	+	+
4.	—	—	+	+
5.	—	+	+	+

It will, thus, be seen that the in vitro results obtained with pentamidine were quite encouraging. Pentamidine in a concentration of 0.1 mg. per ml. of the medium inhibited the growth of *Madurella mycetomi*. Stilbamidine does not hold any promise as an effective chemo-therapeutic agent against *Madurella mycetomi*. However, encouraging the in vitro results with Pentamidine may be, the in vivo results are very important as tissue factors influence chemotherapeutic activity. For example, in the case of tuberculosis it is a puzzling fact that drugs which are powerfully bacteriostatic for tubercle bacilli in vitro and therapeutically effective in vivo against other microbial agents, are of little if any value in the treatment of human tuberculosis; the sulfonamides and chloramphenicol, are examples of this discrepancy. On the other hand, while it is relatively easy to arrest the progress of tuberculous infection with streptomycin, para-amino-salicylic acid, or isoniazid, even prolonged treatment with these drugs often fails to sterilize necrotic lesions. Obviously, there are many factors of the in vivo environment which effect profoundly the antimicrobial activity of drugs. It is, therefore, only after the in vivo tests can a final report be given about the efficacy of a drug. Material for clinical trials has just been received through the courtesy of Messers May & Baker (India) Ltd., New Delhi, who also very kindly provided pentamidine and stilbamidine for the in-vitro tests. The material thus made available will be put to clinical trials in suitable cases of Maduromycosis and a report will be made as soon as any conclusions can be drawn.

My thanks are due to Professor R. M. Kasliwal, Dean and Principal, S.M.S. Medical College, Jaipur, for allowing me to publish this paper.

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ETIOLOGY OF MADUROMYCOSIS IN INDIA

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Supported by a grant from the Indian Council of Medical Research.

(12.7.1955)

(With 7 figures)

Maduromycosis is a chronic granulomatous process, affecting usually the feet and rarely the hand or other parts of the body. It is characterised by variously sized enlargements in the cutaneous surface which eventually give rise to intercommunicating sinuses and fistulae and is associated with the formation of variously coloured granules caused by a variety of filamentous fungi. These fungi belong to different species and genera, which enter the body invariably through traumatic inoculation.

Because "Foot" is the most common site, the disease was named Madura Foot by Colebrook in 1846, on the basis of the work done by Gill of Madura. Most workers, however, do not feel happy about this terminology since the disease occurs in other parts of the world and attacks other regions of the body. Accordingly some workers are of the opinion that "Madura Foot" is not well chosen and should be replaced by the more acceptable term "Mycetoma" created by Carter (1862).

Chalmers & Archibald (1916) used the term Maduromycosis, as caused by *Madurella*, to distinguish the lesion from those caused by Actinomycetes. There is some justification for such a differentiation. Although the lesions may be identical clinically, they differ in that in Actinomycosis or Nocardiosis they may be easily transmitted from one area to another being in many instances systemic in their spread. The lesions of Maduromycosis, caused by *Madurella*, remain fairly well localized, invariably to the sites of trauma and spread usually only by contiguity. Maduromycosis is considered to be a subcutaneous mycotic infection with no evidence of systemic spread. From the histological standpoint also there is a definite distinction of one from the other. On the other hand, to adopt the term Maduromycosis would mean that for each genus causing the disease for which there is no existing terminology a new term would have to be created. Such an increase in terminology would lend more confusion to an already complicated subject. In view of this, it would seem best to adhere to the term Mycetoma and refer to the lesion as Mycetoma caused by the involved organism.

Mycetoma is a pathological condition which is caused by two distinct groups of organisms (1) the true fungi and (2) Fungi-like Bacteria i.e. the organisms of the Actinomycetes group. In a general way, therefore, it will be more informative if we qualify the term Mycetoma by adding a word signifying the type. We now say Actinomycotic Mycetoma and Maduromycotic Mycetoma depending upon whether it has been caused by the Actinomycetes or the true fungi. The following thirty organisms have been isolated and alleged to be the etiologic agents of Maduromycosis:

I. Class Ascomycetes

A. Genus: *Allescheria*

1. *Allescheria Boydii* Shear, 1921.
(*Monosporium apiospermum*)

B. Genus: *Aspergillus*

I. *Aspergillus Bouffardi* Brumpt: 1906.

C. *Genus: Sterigmatocystis*

I. *Sterigmatocystis nidulans* var. *Nicollei* Pinoy, 1906.

D. Genus, *Penicillium.*

I. *Penicillium mycetogenum* Mantelli and Negri, 1915.

II. Class Fungi Imperfecti

A. Genus: Madurella

1. *Madurella mycetomi* (Laveran) Brumpt. 1950
2. *Madurella Bovoi* Brumpt, 1910.
3. *Madurella Oswaldoi* Parreiras Horta, 1919.
4. *Madurella Ramiroi* Piraja da Silva, 1919.
5. *Madurella Tabarkae* Blanc and Brun, 1919.
6. *Madurella Tozeuri* (Nicolle and Pinoy) Pinoy, 1912.
7. *Madurella americana* Gammel, 1926.
8. *Madurella Lackawanna* Hanan and Zurett, 1938.
9. *Madurella Ikedai* Gammel, 1927.
10. *Madurella grisea* Mackinnon et Al, 1949.

B. Genus: *Indiella*

1. *Indiella Mansoni* Brumpt, 1906.
2. *Indiella Reynieri* Brumpt, 1906.
3. *Indiella Brumpti* Piraja de Silva, 1922.

C. Genus *Glenospora*

1. *Glenospora Khartoumensis* Chalmers and Archibald, 1916.
2. Glenospora Semoni Chalmers and Archibald, 1917.

D. Genus: *Monosporium.*

I. *Monosporium apiospermum* Saccardo, 1911.
(*Allescheria Boydii*)
(*Monosporium scleriotiale*)

E. Genus: *Cephalosporium.*
  1. *Cephalosporium recifei* Leão and Lôbo, 1934.
  2. *Cephalosporium falciforme* Carrión, 1951.
  3. *Cephalosporium granulomatis* Weidman and Kligman, 1945.

F. Genus: *Phialophora.*

I. *Phialophora Jeanselmei* (Langeron) Emmons, 1945.
(*Torula Jeanselmei*)
(*Torula Bergeri*).

Maduromycosis is thus caused by a large number of organisms perhaps the largest number which cause any single infection.

Early studies of the subject (ANDLEIGH 1951) showed that in the tissues the organism was seen in the form of conglomerate masses i.e. the granules. Surrounding the granule, which was composed mostly of fungus cells, was the substance known as radiate material, which was eosinophilic. The reaction of the tissues to the invasion of the fungus was found to be a gradual progression from a granulomatous process to a pyogenic one. In the earliest form the fungus, which at this stage consisted of short filaments only, was engulfed by macrophages or multinucleated giant cells. As the filaments increased in number and size, the number of giant cells also increased producing a distint granulomatous process. Further on with the formation of granule polymorphonuclear reaction set in and with the maturation of the fungus granule the giant cells no longer formed part of the reaction and were replaced by a large number of polymorphonuclear leucocytes forming a pyogeinc mass engulfing the granule.

In the hematoxylin and eosin stained sections these granules appeared as light brown. In the stage in which mature granules were present the filaments or hyphae were seen in very small numbers. The filaments had become converted into round or avoid cell groups. These measured about 6—10 microns in diameter. These chlamydospores were seen clearly with the gram stain, but were stained more brightly red, with the periodic acid Schiff Reagent. The granule in the mature form was thus composed of a central mass of segmented branched hyphae 2—4 microns in diameter and throughout the granule were seen many large chlamydospores or hyphal swellings.

In the bloodstreaked pyogenic discharge and in the sinus scrapings, the organism was seen either in masses of small dark brown granules measuring approximately one millimetre in diameter or in the form of bits of older branching hyphae 2—4 microns wide and about 12—18 microns in length.

The organisms causing Maduromycosis in India have not been studied fully in the past. We do not know as to how many genera and species of organisms are responsible for causing Maduromycosis in India. The methods of culturing them and their sensitivity to any chemotherapeutic agents have been studied little or not at all. Investigations have been carried out with a view to elucidate these points. Altogether fifteen cases of Maduromycosis came under observation during the period in which this work was carried out. From these fifteen cases, no strains could be isolated from three cases and in four cases the patients did not cooperate in giving sufficient material for culture and remained under observation for a very short time only. Strains were isolated from eight cases and these eight strains were studied. Among these eight cases there was an interesting case of Mycetoma of the forearm. Very few cases of Mycetoma of parts other than the foot have been reported in the past. In some of the cases reported in the past cultural examination was not done and it is difficult to say definitely if these cases of Mycetoma were of the Maduromycotic type. If we do not consider those cases in which a diagnosis was not confirmed by cultural examination, only five cases have been reported up to now and ours was the sixth case. A brief description of these cases is given below in a tabular form for ready reference.

Name & Year.	Country	Age & Sex.	Duration of the Lesions	Location of the Lesions	Colour of the granules	Organisms isolated
Nicoll & Blanc 1920	Tunisia	19 Female	15 years.	Left hand & Right foot.	Black	*Madurella sp.*
Puestow 1929	U.S.A.	19 Female	6 years	Nose, cheek, right ear, right forearm	Black	*Aspergillus nidulans.*
Symmers & Sporer 1944	U.S.A.	67 Male	7 years.	Right hand.	Black	*Phialophora jeanselmei*
Myerding & Evert 1947	Mexico	39 Male	17 years	Left hand	Black	*Madurella sp.*
Moore 1953	U.S.A.	64 Male	15 years	Right hand and arm	Black	*Madurella ikedai* (?) *M. mycetomi, Madurella americana*)
Sharma & Andleigh 1954	India	20 Male	6 years	Right fore-arm	Black	*Madurella mycetomi*

Culture

Granules were washed in several changes of sterile saline to get rid of the contaminants as much as possible. They were then transferred to a small quantity of sterile broth and crushed into small particles making a homogenous suspension of fine particles. Material from the suspension was then inoculated on the following media:

1. Sabouraud's glucose agar.
2. Malt Tellurite.
3. Brain Heart Infusion Agar with 30 units of penicillin and 40 units streptomycin.
4. Brain Heart Infusion Agar with 25 per cent blood and with pencillin and streptomycin.
5. Brain Heart Infusion liquid medium with penicillin and streptomycin.
6. Thioglycollate liquid medium.

The material was inoculated on two SABOURAUD'S glucose agar slants one of which was put up at 37° C and the other at Room Temperature. On primary inoculation of the material directly taken from the patient very poor growth appeared on the SABOURAUD'S glucose agar either at 37° C or at room temperature even after prolonged incubation. On subculturing the growth from other media to SABOURAUD'S medium colonies appeared in about seven days. The colonies came up with a grayish white mycelium. The mycelial mass was flat, but the central part of the colony was irregularly raised and showed some radiating folds. The mycelia gradually changed its colour from brown to ochre. The mycelial mass of an adult colony was deeply brownish coloured and was covered by an ochre coloured aerial mycelium and the colonies appeared deep brown in colour. A brown to brownish red diffusible pigment was produced. In about three weeks, the whole medium became brownish red in colour. On repeated subculturing this property of pigment production was much lessened. There was a little submerged growth below the surface of the medium.

The vegetative hyphae were cylindrical about 2—4 microns wide. They were septate, the septations resulted in the formation of chlamydospores about 6—12 microns in diameter.

On the Malt Tellurite (Fig. 1) Medium, surprisingly enough, the growth appeared in five days. The growth was dark black with grayish vegetative and greyish-white aerial mycelia, at the periphery. The back of the colony was also dark and diffusion of any reddish brown pigment could not be made out. On repeated subcultures one could notice the appearance of the growth even after 18—24 hours incubation. The microscopic examination of the growth showed the presence of the same mycelia, arthrospores and the chlamydospores.

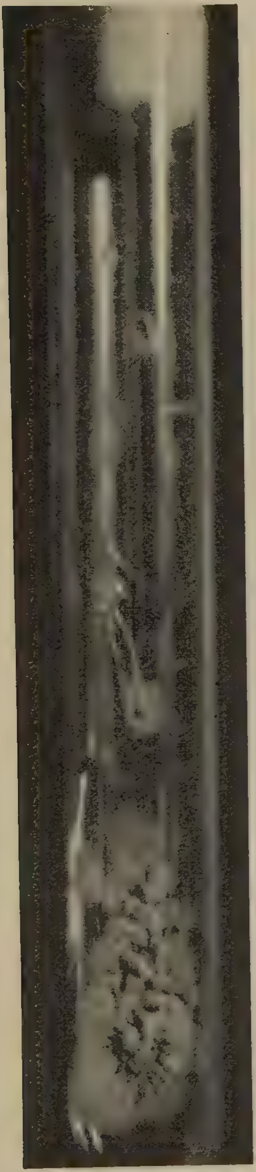

Fig. 1

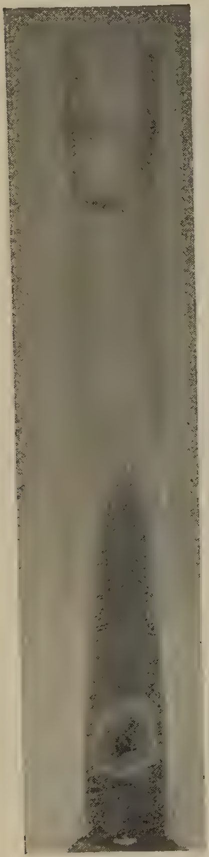

Fig. 2

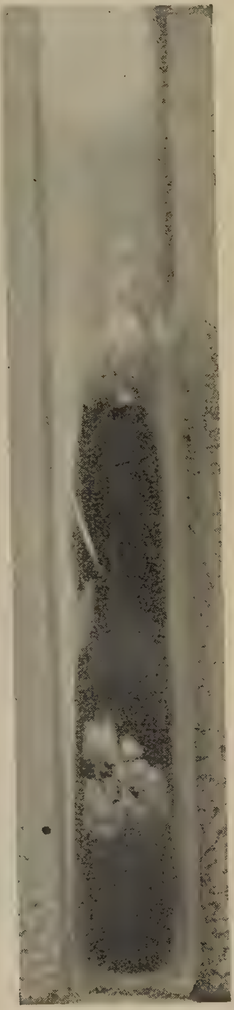

Fig. 3

On plain Brain Heart infusion agar with penicillin and streptomycin (Fig. 2) the growth appeared in about 5—6 days and grew to the size of about one centimetre in diameter in two weeks. At first a somewhat irregularly raised greyish brown mass appeared at the site of primary inoculation. From this central mass irregularly furrowed and convoluted growth radiated outwards. Around this intermediate zone was a zone of about 2 mm width formed by greyish white vegetative mycelia and whitish aerial mycelia. A characteristic feature was the production of pigment. Right from the very beginning an orange brown pigment could be seen diffusing into the medium at the back of the colony. The pigment production was best at room temperature and in about two weeks time the whole medium became orange brown in colour. However, this property of pigment production gradually diminished on repeated subculturing and after about three months, in which subcultures were repeated about six times, very little pigment production was noticed. Again pigment production was less marked on this medium than on Sabouraud's glucose agar. The microscopic appearances of the growth, in a lactophenol mount, were similar to those of the growth from Sabouraud's glucose agar.

On Brain Heart Infusion agar with 10 to 20 per cent of blood and with penicillin and streptomycin (Fig. 3) the addition of blood did not seem to stimulate the growth. The growth neither appeared earlier nor was it more profuse. The growth appeared in about 7—8 days and was from dark brown to almost black in colour. It did not show the same three zones distinctly as were seen on the Brain Heart Infusion agar without blood, but still they were apparent. This was due to the fact that the whole growth was much darker in colour and the outer most flat area which formed a grayish white zone on plain Brain Heart infusion was of a dark brown colour on this media. Further, the central irregularly raised portion at the site of primary inoculation did not appear on this media regularly. It is probable that the presence of the pigment at the back of the primary inoculum on plain Brain Heart agar made it very much more prominent than it would have been otherwise. Of course, the production of pigment cannot be appreciated on a media containing such large amounts of blood especially when the pigment produced is of a similar colour. Microscopic examination of the growth on this media in the form of a lactophenol mount showed the presence of mycelia and their spores and few chlamydospores. Hyphal swellings were more prominent in the growth on this media otherwise the picture was similar to that seen in the growth on Sabouraud's media.

On Brain Heart Infusion liquid medium (Fig. 4) the growth appears in about 4 days. It appeared in the form of snow flakes floating about in the liquid, the blackish granule particle forming a sort of a central nucleus from which this snow flake-like growth of greyish white tufts of mycelia spread outwards and all around the black central nucleus. These snow flakes were irregular in size and in

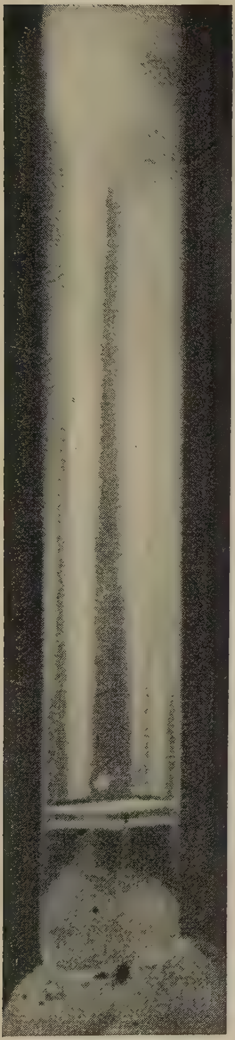

Fig. 4

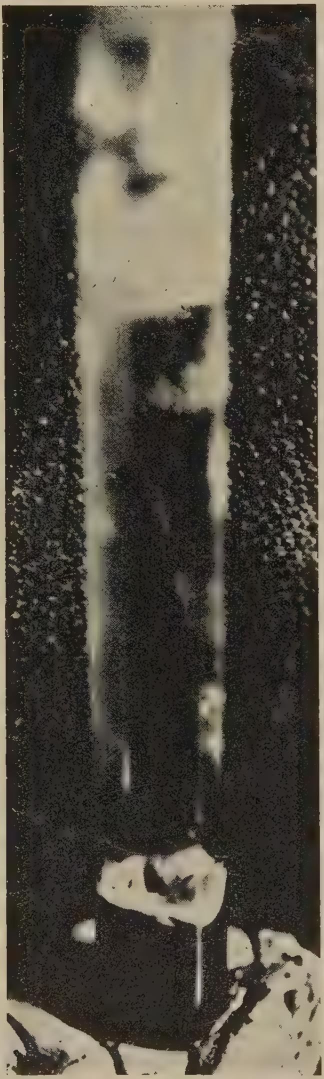

Fig. 5

density and tended to settle down at the bottom of the tube in the early stages. Later, as the growth spread, it gradually filled up the medium in which it remained suspended and floating. Pigment production in this medium was less marked than on Sabouraud's medium and it took about 15—18 days for the straw coloured fluid medium to become an orange brown colour.

The microscopic examination of the growth in the form of a lactophenol mount showed wide branching septate mycelia about 3—5 microns wide. These were segmented to form somewhat rectangular or oval arthrospores about 5—8 microns long and 4—6 microns wide. Few chlamydospores were also present and stray sclerotia were seen.

On Thioglycollate liquid medium (Fig. 5) the growth appeared in about 5 days as an irregularly matted brownish gray mold-like velvety growth, in the form of a pellicle on the surface of the medium. There was also a little growth in the upper 1/3 of the medium, continuous with the surface growth. Pigment production was much more marked on this medium than on the Brain Heart Infusion liquid medium probably due to the microaerophilic conditions produced. Within a few days of growth the upper half of the media became a deep red colour.

Fermentation Reactions

On ordinary sugars growth was scanty and appeared in about 8 days time. There was no fermentation of any sugar and no gas was produced. In the ordinary sugar media the growth was most marked on the surface and in the upper part of the media. Except for the increase in the growth no other change was noticed on further incubation. On sugars prepared in the Brain Heart Infusion liquid medium, the growth was exactly as seen in the simple Brain Heart Infusion liquid medium. No sugars were fermented and no gas was produced. The growth appeared earlier, in about 5—6 days, and was in the lower part of the medium. The medium also became brown orange in colour.

Loeffler's Serum

On the Loeffler's medium growth appeared in about 7—8 days. The growth was similar to that seen on Brain Heart Infusion agar. There was some digestion of the medium.

Starch Medium

There was poor growth on this medium and there was hydrolysis of starch to some extent.

Animal Inoculation

Four Rabbits and four Guinea pigs were inoculated with a heavy inoculum subcutaneously for the purpose of studying the various stages in the formation of the granuloma and their mode of spread. No lesions could be produced in any of the animals.

Classification of the Organism

According to DODGE (1935) the genus *Madurella* is placed in the tribe Toruleae. The Toruleae are characterised as follows "Mycelium usually dark coloured, no differentiation of sporophores, multiplication by arthrospores and chlamydospores, rarely sprouting. The genus *Madurella* is a difficult one to classify properly since the morphology of the various species included in it has not been adequately studied." DODGE lists eight species. Recent studies, however, have shown that some of them might well be synonymous with others.

The genus *Indiella* appears to be clearly related to *Madurella*. Here the organism produces colourless or white granules, forming a white thallus and is consequently included in the Mucedineae. CIFFERI & REDAELLI (1941) combine the species of *Madurella* and *Indiella* in one genus *Madurella*. Most of the workers, however, classify *Madurella* and *Indiella* as distinct genera.

Further classification of the genus *Madurella* is still in a state of confusion. The genus *Madurella* was created by BRUMPT (1905) using as the type species, the organism described as *Streptothrix mycetomi* by LAVERAN (1902). In both instances the species and genus differentiation was based on the observation of granules and not on actual cultures. In 1911 CHATTERJEE isolated a culture belonging to the *Madurella sp.* According to MACKINNON (1951) the strain isolated by CHATTERJEE was very similar to *Madurella grisea*, although CHATTERJEE did not name his strain. BRAULT (1911) isolated and described *Madurella mycetomi* in culture and NOC & JOUNNE (1922) published a detailed cultural description of the fungus. According to BRAULT, *Madurella mycetomi* was described as follows.

"Mycelium grayish white, yellowing with age, darkening sugar culture mediums, oidia of variable dimensions 2—5 microns, sclerotia black sterile 0.5 mm in diameter found in the interior of the Culture media. Able to invade in man skin, bone, muscle and the adjacent tissue producing a Mycetoma with black granules. The granules are small hard round more or less verrucous, with a morphology simulating that of the sclerotia found in the culture. Animal inoculations are negative".

This description appeared inadequate and was, therefore, enlarged by YAZBEK (1920), NOC & JOUENNE (1922), GAMMEL (1927) and recently by MACKINNON (1951). By combining the characteristics described by these various investigators *Madurella mycetomi* has been described as follows *Madurella mycetomi* (Laveran) Brumpt (1905). In tissues there are produced hard dark brown to black, discrete or conglomerate granules round irregular or verrucous in form, measuring approximately 0.5 mm in diameter. Granules composed of brown septate hyphae measuring 2—5 microns in diameter with hyphal swellings measuring approximately 3—15 microns in length and chlamydospores both intercalary and terminal are also present, measuring 7—12 microns in diameter, but may become approximately 25 micrones. An interstitial brown substance may be present in spaces among hyphae.

On Sabouraud's glucose agar, the organism produces a greyish white mycelium which becomes yellow and then brown with age and covered with a yellowish brown aerial mycelium. The colony attains a size of 1—2 cms. in diameter. The center of the colony becomes verrucous and raised and develops radiating furrows with age. On various carbohydrate media a brown pigment diffuses into the substrate. The vegetative hyphae are septate, irregular or regular with hyphal cells approximately 1—5 by 7—26 microns. Chlamydospores are either terminal or intercalary measuring approximately 7—20 microns in diameter with some measuring up to 26 microns or even larger. Sclerotia measuring 0.5 mm. are present in Sabouraud's proof medium and in potato water and peptone broth, some 50 times within the medium. The culture may lose this property. The optimum temperature for growth is approximately 37° C. Gelatin may or may not be liquified, milk is usually not coagulated.

The organism produces in man Mycetoma with involvement of the skin, muscles, bones and joints.

The discrepencies in the description reported by Brault, Yazbeck and Noc & Jouenne might mean a different interpretation of the same species or perhaps even different species. Mackinnon for example lists the chlamydospores as measuring 7—20 microns where as Brault considers them to be 7—12 microns and Noc & Jouenne further describes moniliform filaments where as Yazbek described fine regular filaments.

In the United States there have been described three species of *Madurella: M. lackawanna, M. americana* and *M. Ikadai*. Their characters are as follows:

M. lackwanna.

Black granules, brown chlamydospores intermingled with thick-walled septate mycelium, nodular organs present, brown raised colony thrown into cereberiform folds covered with dawny mycelium, grows very slowly and poorly on Sabouraud's medium. Grows best on liver infusion Sabouraud's agar. Filaments rather straight, usually alternately branching, granular from 1—5 microns in diameter, early formation of chlamydospores from 15—30 microns in diameter, occassional oidiospores formation of nodular organs, gelatin not liquified. Proteins digested in milk.

Madurella americana (*Gammel*).

Mycelium grayish white, then old yellowish brown and darkening certain sugar mediums. Hyphae subhyaline or brown and granular varying in dimensions from 1 to 5.2 microns, according to the percentage or type of sugar chlamydospores numerous especially in SABOURAUD'S glucose broth (hanging drop). Arthrospores with square cut ends rare. Optimal medium 7 % glucose agar. Growth on SABOURAUD'S agar none or very poor, tomentous or cereberiform on Grutz agar, sclerotia of 1 mm. in diameter on surface of gelatin and SABORAUD'S preservative agar, liquefies gelatin, ferments milk, thermophilic pigment formation, aboundant in glucose maltase, dextrin and galactose, moderate in levulose, poor in saccharose and lactose none or very poor in inulin broth. Animal inoculation negative.

Madurella Ikedai (*Gammel*).

Mycelium grayish white later light brown darkening certain sugar mediums. Hyphae hyaline or subhyaline, rarely granular varying in diameter from 1.5 to 5 μ. Chlamydospores numberous only in SABOURAUD'S glucose broth in all liquid mediums. Many hyphae break up into chains of lemoniform or round spores. Acuminate light brown colonies on SABOURAUD'S and GRUTZ' agar. Sclerotia numerous on surface and in depth of SABOURAUD'S preservative medium, liquifies gelatin, ferments milk, is thermophilic, pigment production abundant in glucose, galactose and maltose, moderate in mannite and levulose, poor in dextrin, poor or absent in inulin, absent in saccharose and lactose broth. Not pathogenic for rabbits nor guinea pigs. Isolated by IKEDA from Maduromycosis with black grains in Minneapolis.

M. lackawanna is sufficiently different from all other species of the genus and from the organism described here and thus need not be considered any more in the present discussion. The remaining two, *M. americana* and *M. Ikedai*, however, need further consideration. According to GAMMEL both species are fairly slow growers, both produce white mycelium which becomes yellowish brown and both produce sclerotia on the surface of SABOURAUD'S preservative agar. The filaments of both species vary from 1—5 microns in diameter. *Ikedai* forms numerous moniliform oidia, whereas *M. americana* shows rare formation of oidia. Chlamydospores appear late in *M. americana* but area bundant in *M. Ikedai*. In spite of these minor differences the microscopic morphology of *M. americana* and *M. Ikedai* is very similar and resembles closely the morphology of *M. mycetomi* as illustrated by BRUMPT. MACKINNON (1951) has, therefore, classified *M. americana*, *M. Ikedai* and *M. mycetomi* under one species, *M. mycetomi*. GAMMEL now feels that *M. Ikedai* is similar to *M. americana* and that both perhaps should now be known as *M. americana*.

Madurella grisea (*Mackinnon*).

Another important species of the genus *Madurella* is *grisea*. *Madurella grisea* has been described by MACKINNON as follows: "On SABOURAUD'S glucose agar hard colonies built by an almost black folded mycelial mass covered by a white grayish pubescence. The pubescence hides the folds of the mycelial mass, but some strains produce scarce and shorter aerial mycelium and consequently the colonies are darker and the folds are more visible. The medium becomes brown around the colonies. In CZAPEK'S liquid medium spherical, submerged colonies of hyaline mycelium with dark center are produced, later on a veil may cover the surface. Hyphae may be moniliform (6—4") or cylindric (2—3"), fuligenous, branched and septate. These give rise to the slender almost hyaline young aerial hyphae. The cylindric mycelium predominates in most of the strains, but in one strain the moniliform hyphae predominate. This strain produces scarce pubescence and the folding of the mycelial mass is quite visible in the giant colonies. Intercalary and terminal thick walled chlamydospores (4—7 × 3—4) are very scarce and some strains failed to produce resting spores.

The following sugars are assimilated: glucose, maltose, galactose and sucrose, lactose is not assimilated. The following nitrogenous compounds are assimilated: peptone, asparagine, urea, sodium and potassium nitrate and ammonium sulphate, optimal temperature 30° C. weak and very slow proteolysis of gelatine and milk, very weak and very slow proteolysis of coagulated serum. Starch is hydrolyzed.

The strains isolated from the 8 cases were all *Madurella mycetomi*. It appears that *M. mycetomi* is the commonest infecting organism in this part of India. The particular features brought out by these studies in respect of the growth characters etc., of *M. mycetomi* are as follows:

1. *M. mycetomi* takes about three weeks to grow on the routine Sabouraud's medium, but on the Brain Heart infusion agar and Brain Heart Infusion broth growth takes place within 4—5 days and after repeated subculturing the growth may appear even within 48 hours.

2. The best procedure of getting pure growth in a short time has been found to be as follows:

The granules are washed repeatedly in several changes of sterile normal saline, then rapidly washed with a quick change of 30 % alcohol. It is then again washed with two changes of sterile normal saline and than with two changes of Brain Heart Infusion liquid medium containing penicillin, streptomycin and actidione. The granules are then crushed thoroughly in the Brain Heart infusion liquid medium so as to form a suspension of fine particles and this is then inoculated on to the Brain Heart Infusion agar with 30 units of penicillin and 40 units of streptomycin and to the Brain

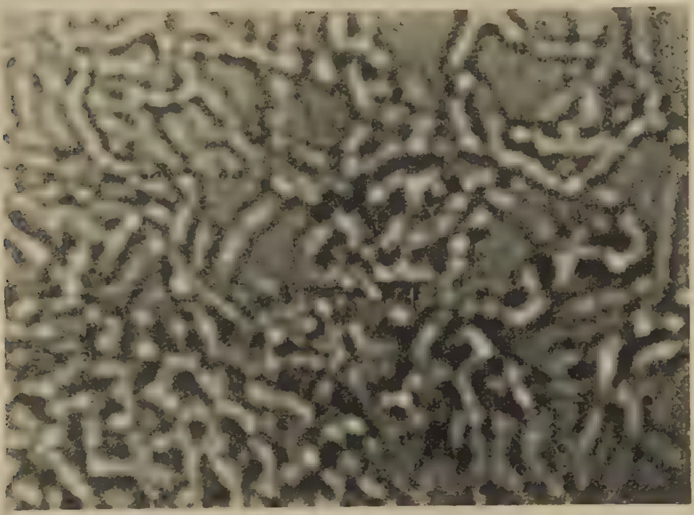

Fig. 6. Madurella Mycetomi Brain Heart Agar 10 days growth at 37 C × 360.

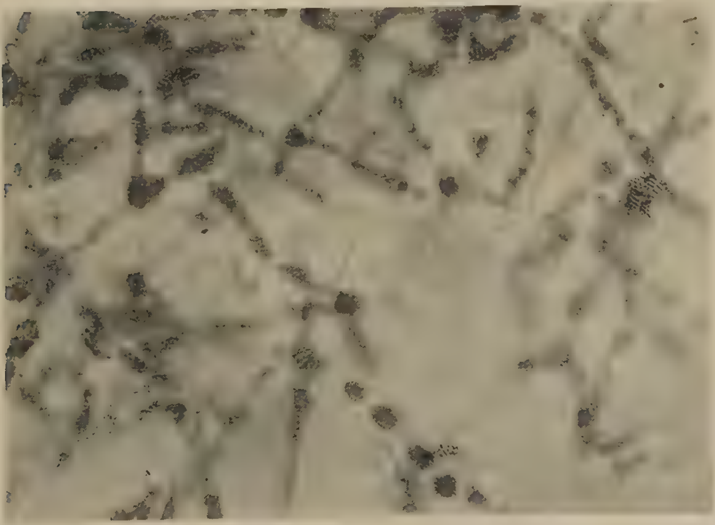

Fig. 7. Madurella Mycetomi Brain Heart liquid Media, 10 days growth at 37 C. Note the chlamydospores × 360.

Heart infusion liquid medium containing 30 units of penicillin and 04 units of streptomycin per ml.

3. To the description of *Madurella mycetomi* given by Moore (1954) the following observations are well-worth being added. On Brain Heart agar growth appears in about 5—6 days and grows to the size of about one centimeter in diameter in about two weeks. The growth as such assumes a form of having been formed of three zones, a central zone of raised black verrucous mass surrounded by a gray to brown radiating ridges and outside this a zone of whitish aerial mycelia. Brown pigment is produced. On Brain Heart Infusion liquid medium, with penicillin and streptomycin, the growth appears in about 4 days in the form of snow flakes with a blackish central point. Pigment is produced. On Thioglycollate medium the growth appears in about 6 days as an irregular matted brownish gray mold-like pellicle right on the surface of the medium. Pigment production is more marked on this medium.

4. Moore (1954) classified the fungus he isolated from a case of Mycetoma of the hand and arm temporarily as *Madurella Ikedai* Gammel (1927) possibly synonymous with *M. americana* and *M. mycetomi*.

From a study of the characters of the various species of the genus *Madurella* it appears that there are only three distinct species of the genus i.e.

1. *Madurella mycetomi.*
2. *Madurella grisea.*
3. *Madurella lackawana.*

The rest of the species i.e. *Madurella americana, Madurella Ikedai, M. Bovi, M. Oswaldai, M. Ramiroi, M. Tabordde,* and *M. Tonzeuri* are probably all synonymous of *Madurella mycetomi*. This probably explains why *Madurella mycetomi* has been found to be the commonest causative organism of Mycetoma of the foot in India.

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SPOROTRICHUM CEREBRIFORME NOV. SPEC.

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(Mit 2 Figuren)

Während der zweiten schleswig-holsteinischen Mikrosporieepidemie, die im Winter 1945/46 begann, und deren wechselvollen Verlauf wir bis 1953 beobachten konnten (Kleine-Natrop [1, 2]), wurden von der Hautklinik der Christian-Albrechts-Universität Kiel umfangreiche Reihenuntersuchungen in den epidemiegefährdeten Kreisen des Landes vorgenommen. Die rationelle Durchführung solcher Reihenuntersuchungen mit einer möglichst hohen Sicherheitsquote der Erstdiagnosen ist zweifellos von der Verwendung geeigneter Fluoreszenzlampen abhängig (Kleine-Natrop [3]). Innerhalb eines großen Untersuchungsgutes finden sich aber immer Fälle, in denen die Grenzen der Fluoreszenzdiagnostik bei der Mikrosporie deutlich werden.

Über eine derartige Situation sei im folgenden kurz berichtet: Im Stirnhaarbereich eines 16-jährigen Mädchens, das in der seit langem mikrosporieverseuchten Umgebung eines Jugendheimes lebte, fand sich ein schwachgrün-fluoreszierender, rundlicher, pityriasiformer Herd von etwa 3 cm Durchmesser. Dieser veränderte Kopfhautbezirk war ohne Woodlicht mit bloßem Auge gerade eben zu erkennen, sofern man vorher auf ihn aufmerksam geworden war. Mit dem tastenden Finger war er indessen wegen seiner ausgeprägt seborrhoisch-pityriasiformen Note gut wahrzunehmen und zu umgrenzen. Die Haare im Herdbereich waren von normaler Festigkeit und schienen nicht an dem Krankheitsgeschehen beteiligt zu sein. Im Gegensatz zu den Schuppen regte das Anstrahlen mit dem Woodlicht bei ihnen keine Lumineszenz an. Obwohl sie im Hinblick auf den sehr derben Kamm, den das Mädchen benutzte, nicht ausgeschlossen schien, war eine vorausgegangene oberflächliche Verletzung der Kopfhaut anamnestisch nicht zu erheben.

Mit Rücksicht auf die Fluoreszenz des Herdes, die zwar nicht typisch war, aber sehr an Mikrosporie erinnerte, wurde die Primärdiagnose Tinea microsporica gestellt. Als unterstützende Argumunte kamen die Häufigkeit der pityriasiformen Abart der klassischen Mikrosporie in Schleswig-Holstein, sowie die Tatsache hinzu, daß in dem Jugendheim, in dem das Mädchen lebte, seit Jahren Mikrosporieerkrankungen vorgekommen waren. Der positive Pilznachweis bei der späteren mikroskopischen Untersuchung schien die

Diagnose zu bestätigen; erst die Kultur auf GRÜTZ-Agar zeigte dann, daß es sich bei dem vermutlichen Erreger nicht um ein Microsporum handelte, sondern daß vielmehr eine Sporotrichum-Art vorliegen mußte.

Die Frage nach dem ursächlichen Zusammenhang solcher oberflächlicher Veränderungen der Kopfhaut mit dem Pilzbefall ist nicht leicht zu beantworten. Obschon Sporotrichosen alle Gewebe in Mitleidenschaft ziehen können (GOUGEROT, FUHS) und sowohl primäre als auch sekundäre, ausschließlich epidermale und epidermido-cutane Formen (Dermo-Epidermitis, Lichen) unter dem Erscheinungsbild einer banalen Pityriasis bekannt sind (GOUGEROT, BENEDEK), erscheint in unserm Fall Zurückhaltung geboten. Der Pilz wurde bisher nur bei einer Patientin gefunden und konnte zweimal aus Kopfhautschuppen, dagegen nicht aus den Haaren isoliert werden.

Zur Information über die Pathogenität dieser Sporotrichum-Art wurden Inokulationsversuche bei sechs männlichen Ratten vorgenommen. In der ersten Serie wurden den Tieren 10 ccm einer Kulturaufschwemmung intraperitoneal injiziert, in der zweiten große Kulturpartikel operativ in die Bauchhöhle gebracht. Alle Ratten zeigten keinerlei klinische Krankheitszeichen und überlebten. Die mikroskopische Untersuchung der Organe ließ sowohl entzündliche Veränderungen, besonders die charakteristische Orchitis, als auch positive Pilzbefunde vermissen. Um die Möglichkeit einer Hautpathogenität gesondert zu prüfen, wurden fünf Meerschweinchen nach Depilation und oberflächlicher Skarifikation in üblicher Weise mit Kulturpartikeln beimpft. Diese Impfungen gingen jedoch ebenfalls nicht an. Natürlich schließen diese negativen Ergebnisse eine Pathogenität des Pilzes für den Menschen noch nicht aus. Bisher ließ sich aber nicht klären, ob der Pilz, der auf den Hautveränderungen gefunden wurde, als primärer oder sekundärer Parasit, oder nur als apathogener Contaminant zu werten ist.

Bei der mykologischen Prüfung der Sporotrichum-Art ergaben sich folgende Daten:

Makroskopisches Bild nach 32 *Tagen Bebrütung im Brutschrank bei* 25° C.

(DK = Diameter der Kultur — R = Ridgeway, Color standards and nomenclature)

Czapek-Agar — DK 25 mm. Vom Impfstück ausstrahlende moosartige, hyaline, verzweigte Myzelbündel. Die Kultur ist flach, ein Luftmyzel kaum ausgebildet. Unterseite der Kultur hyalin. Etwas säuerlicher Geruch.

Pepton-Agar — Dk 23 mm. Die Kultur ist flach, weiß, hell-braungelb angehaucht. Hyphen stehen dichtgedrängt. Die Unterseite ist wie die Oberseite gefärbt.

Bierwürze-Agar — DK 14 mm. Die Kultur ist hochgewölbt. Sie bildet einen festen Hügel und ist besonders in der Mitte mit weißem oder durch die Feuchtigkeit gelbbraunem („Mars Yellow", R III), kurz-wolligem Luftmyzel bedeckt. Am Rande ist das Luftmyzel geringer, dadurch wird die gelbbraune Farbe besser sichtbar. Die Unterseite ist ebenfalls gelbbraun. Kein spezifischer Geruch.

Sabouraud-Maltose-Agar — DK 23—27 mm. Die Kultur ist ziemlich flach und zeigt innerhalb eines 2—3 mm breiten, flachen, feuchten, grau-weißen Saumes, der mit kurzem, feuchtem, hyalinem Luftmyzel bedeckt ist, mehr oder weniger deutliche radiäre Furchen. In der Mitte können feine, hirnartige Windungen auftreten. Die Farbe ist creme („Light Buff", R XV) und wird zum Rand hin heller. Die Unterseite ist gefärbt wie die Oberseite.

Sabouraud-Glukose-Agar — DK 28—33 mm. Die Kultur ist ziemlich flach, in der Mitte ein wenig eingesunken, kurz-weißfilzig und hat einige radiäre Furchen. Das Substratmyzel ist feucht und cremefarben („Light Buff", R XV). Die Unterseite zeigt die gleiche Farbe, aber einen konzentrischen, gelben („Napels Yellow", R XVI) Ring. Kein spezifischer Geruch.

Haferflocken-Agar — DK 28 mm. Die Kultur ist flach, feucht, mit konzentrischen Zonen. In der engeren Peripherie des Impfstückes ist sie grauweiß, daran schließt sich eine 4 mm breite, feuchte, gelbbraune („Chamois", R XXX) Zone. Unmittelbar innerhalb der 1 mm breiten Randzone kann sich ein Luftmyzelring von 2 mm Breite ausbilden. Kein spezifischer Geruch.

Maismehl-Agar — DK 25—27 mm. Die Kultur ist flach, feucht, grauweiß und hat in der Mitte spärliche Fläumchen Luftmyzel.

Kartoffel-Agar — DK 13—20 mm. Die Kultur ist ein wenig gewölbt, feucht, mit kurzen, abstehenden Hyphenbündeln, cremefarben („Light Buff", R XV). Unterseite von gleicher Farbe. Der Geruch ist würzig.

„Littman-oxgall"-Agar — DK 10—12 mm. Die Kultur ist hochgewölbt, mit winzigen oder auch gröberen hirnartigen Windungen, blaugrau („Light Dull Bluish Violet", R XXXVI), am Rande, wo ein sehr kurzes Luftmyzel wächst, hellgrau. Die Unterseite ist „Madder Blue" (R XLIII), wird zum Rand hin heller.

Kartoffel + Glyzerin — Sehr üppiges Wachstum. Kultur ist hochgewölbt, stark hirnartig gewunden, unten im Rohr wulstig, feucht, mit spärlichen, kurzen, feuchten, Luftmyzelhyphen. Am oberen Ende des Kartoffelstäbchens ist sie trocken und dicht weiß-filzig. Unten im Rohr cremefarben („Light Buff", R XV), in der Mitte und oben dunkler, „Light-Ochraceous Salmon" (R XV) bis „Ochraceous-Salmon" (R XV) und „Ochraceous

Buff'' (R XV). Die Kartoffel ist nicht verfärbt. Nach 113 Tagen ist die Farbe rosa-orange (,,Light Pinkish Cinnamon'', bis ,,Pinkish Cinnamon'', R XXIX). Der Geruch ist säuerlich.

Möhre — Gutes Wachstum. Die Kultur ist hochgewölbt, feucht und hat ausstrahlende, spitzige Hyphenbündel, die stellenweise weiß und trocken werden. Die Farbe ist ,,Ochraceous Buff'' (R XV), nach 113 Tagen ,,Cinnamon'' (R XXIX). Keine Verfärbung der Möhre, kein spezifischer Geruch.

Czapek-Lösung — Sehr geringes Wachstum. Ein weißes Fläumchen auf dem Impfstück unten im Rohr.

Auf keinem der genannten Nährböden bildete der Pilz ein lösliches Pigment.

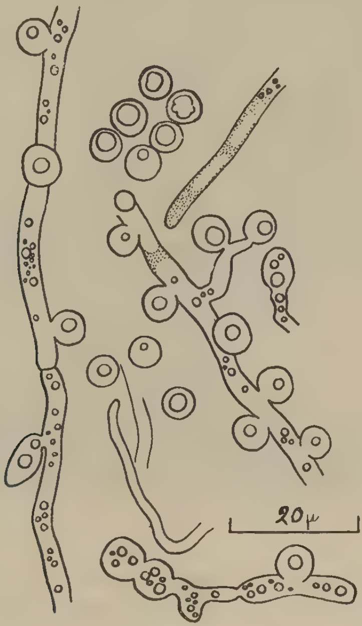

Fig. 1.

Sporotrichum cerebriforme nov.spec.; Mikroskopisches Bild von Myzel und Sporen einer 11-tägigen Kultur auf Sabouraud-Glukose-Agar. Bebrütungstemperatur 25° C.

Mikroskopisches Bild (Abb. 1)

Die Hyphen sind hyalin, verzweigt, haben Querwände in sehr verschiedenen Abständen, mit vielen Öltröpfchen; sie sind dünnwandig, durchschnittlich 2.2—4.5 μ dick. Die meisten Hyphen sind ziemlich gleichmäßig, jedoch kommen auch, z.B. auf Bierwürze- und Sabouraud-Maltose-Agar, aufgetriebene Hyphenzellen vor. Diese sind rund oder oval, liegen einzeln oder in Ketten und werden bis

30 μ dick. Die Konidien sitzen entlang den Hyphen, selten endständig an Seitenhyphen. Sie sind rund, hyalin, glatt und tragen Öltröpfchen, 4.5—6.5 μ.

Einfluß der Temperatur auf die Wachstumsgeschwindigkeit und das Aussehen der Kultur. (Abb. 2)

(Temperaturangaben in Grad Celsius)

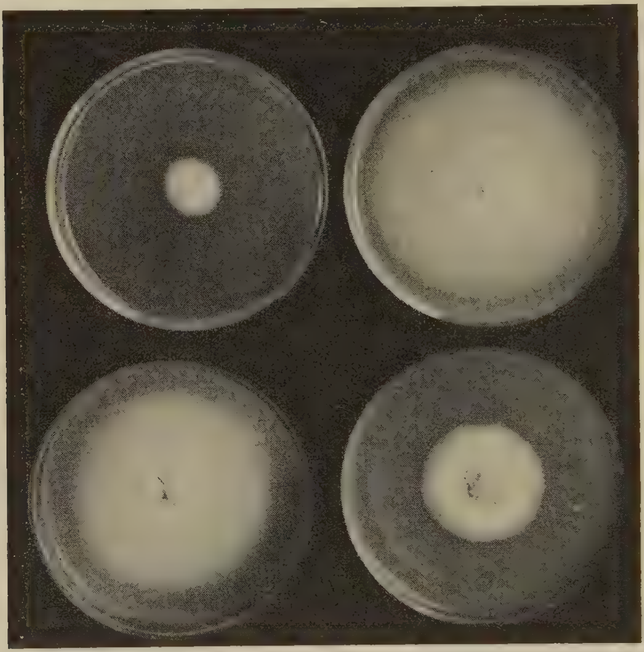

Fig. 2.

Sporotrichum cerebriforme nov.spec. Kulturen in Petrischalen auf Sabouraud-Glukose-Agar nach 45-tägiger Bebrütung bei verschiedenen Temperaturen. Links oben 0° C, rechts oben 15° C, links unten 20° C, rechts unten 25° C.

Die optimale Wachstumstemperatur, die bei ungefähr 20° liegt, wurde am Radiärwachstum von Kolonien auf Sabouraud-Glukose-Agar in Petrischalen (Durchmesser 9 cm, Höhe der Agarschicht 3—4 mm) bestimmt. Kein Wachstum zeigte sich bei 30° und 27°, nur sehr langsames Wachstum bei 0°. Das Temperaturoptimum für die Sporenbildung ist offenbar ein anderes. Bei 25° wurden in den Präparaten keine Sporen gefunden, bei 20° und 0° sehr viele, nur wenige dagegen bei 15°. Die zentrale Faltenbildung ist sehr deutlich in Kolonien, die bei 25° gewachsen sind, weniger deutlich bei 20°. Bei Temperaturen von 0° und 15° bleibt die Kolonie ganz flach. Die Farbe ist bei 25° ,,Light Ochraceous Buff' (R XV), bei 20°

„Cream Buff" (R XXX), bei 15° „Pale Pinkish Buff" (R XXIX) und bei 0° grauweiß.

Die Definition, die LINK 1809 der Gattung Sporotrichum gab, lautet: "Thallus e floccis intricatis decumbentibus aut erectiusculis, ramosis, septatis. Sporidia ubique inspersa, rotundata". Weiter schreibt er noch folgendes: "Caespitibus late effusis truncos caesas aliaque vegetabilia putrescentia investiunt. Varios habent colores, saepe pulcherrimos. Sporidia plerumque globosa, parva aut minuta, varia copia inspersa". Also: Das Myzel liegt flach auf dem Substrat oder richtet sich als Luftmyzel vom Substrat empor. Die Hyphen sind verzweigt und septiert. Die meist runden, kleinen oder sehr kleinen Sporen sind regellos am Myzel angeordnet. Die Farbe der Rasen ist verschieden, oft sehr schön.

Obwohl die Konidien unseres Pilzes ziemlich groß sind, können wir ihn nur in diese Gattung einreihen. Vom bekannten Erreger der Sporotrichose, Sporotrichum schenkii (Hektoen et Perkins) Beurmann et Gougerot unterscheidet er sich ganz erheblich. Dieser hat nämlich außer seitlichen Konidien auch solche, die am Ende richtiger Träger gebildet werden. Von der Sporotrichum-Stufe des Fusarium poae (Peck) Wr., früher Sporotrichum poae Peck, weicht der neue Schimmelpilz durch seinen nicht staubig-spinnwebartigen, sondern derbfeuchten Kulturcharakter, durch die Abwesenheit von Makrokonidien und die kleineren und regelmäßiger runden Konidien ab.

Da sich in der Literatur kein Sporotrichum fand, dessen Beschreibung auf das Bild unseres Pilzes gepaßt hätte, wird dieser unter dem Namen *Sporotrichum cerebriforme* nov. spec. als neue Art aufgefaßt und beschrieben.

Die vollständige Diagnose lautet: Thallus in agaro nutritivo et in Solani tuberosi et Dauci carotae fragmentis cinnamomeus, saepe cerebri modo circumvolutus. Hyphae hyalinae, ramosae, septatae, olei globulos continentes, 2.2—4.5 μ latae, interdum cum cellulis ad 30 μ inflatis. Conidia in hyphis vegetabilibus lateraliter sessilia, levia, globosa, hyalina, olei globulos continentes, 4.5—6.5 μ.

Hab.: Ex cutis capitis hominis squamis in Germania septentrionali isolatus.

Reinkulturen wurden in folgende Sammlungen eingereiht: Centraalbureau voor Schimmelcultures, Baarn, Nederland — American Type Culture Collection, Washington, U.S.A. — Commonwealth Mycological Institute, Kew, Surrey, England. Abgetötete und getrocknete Kulturen wurden an Royal Botanic Gardens, Kew, Surrey, England; Rijks Herbarium, Leiden, Nederland und New-York Botanical Gardens, New York, U.S.A. eingesandt.

Summary

1) Sporotrichum cerebriforme nov. spec. has been isolated from a seborrhoic-pityriasis-like, light-green fluorescent lesion on the scalp of a 16-year old girl.

2) The possible role of this fungus in the pathogenic process could not be established.

3) The fungus was not pathogenic for rats and guinea pigs.

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FREDERICK DEFOREST WEIDMAN
(1881—1956)

IN MEMORIAM

FREDERICK DEFOREST WEIDMAN

While on a vacation in Maine, Dr. WEIDMAN died suddenly on August 30, 1956, ending a career of immense value to the world, and bringing sorrow to the hearts of thousands who knew him personally and through his writings.

Born in Bristol, Connecticut in 1881, he graduated in medicine from the University of Pennsylvania in 1908 and shortly thereafter began the career of teaching and research, which he chose in preference to the practice of medicine in the ordinary sense.

After his interneship in the Delaware Hospital in Wilmington, he became assistant demonstrator in histology and pathology in his Alma Mater and in 1914 was made instructor in pathology. During his service in this capacity the Laboratory of Dermatological Research was founded by a bequest in the will of Prof. DUHRING. WEIDMAN was chosen as assistant director of this laboratory. In 1921 he was made Acting Head of the department of Dermatology and Syphilology and in 1923, Professor of Dermatological Research and Director of the laboratory.

During this period WEIDMAN was also demonstrator in pathology and then Professor of Pathology in the Woman's Medical College of Pennsylvania, and assistant pathologist, then associate pathologist of the Philadelphia Zoological Society, a position which he held for many years.

The great change in FRED WEIDMAN's career was the foundation of the Laboratory of Dermatological Research in 1917 and his appointment as assistant director, thus orienting him toward dermatology. He is distinguished as the first American physician to choose teaching and research in dermatology as his life work. He had acquired a fine foundation in human pathology and parasitology under Professor J. ALLEN SMITH, the eminent parasitologist, who recommended him for the laboratory position, and WEIDMAN also had a consuming interest in our bacteriological and mycological enemies. The mycology of human disease was a comparatively new field at that time, very attractive to WEIDMAN. His energetic work as assistant director of the laboratory had much to do with the acquisition of a nucleus of mycologic, histologic and photographic material that was the foundation of the present extensive collection.

While refreshing his knowledge of dermatology under Professor HARTZELL, his superior in the laboratory, WEIDMAN continued until 1919 the teaching of undergraduate pathology with responsibility for the autopsies and his work as consultant for the Zoological Gardens.

From 1920 until 1947, WEIDMAN was visiting dermatologist at the

Philadelphia Naval Hospital. During the second world war he was consultant in tropical medicine to the Secretary of war.

In the Graduate School of Medicine, University of Pennsylvania he was Vice-Dean, Professor of Dermatology and Syphilology and Chief of the Clinic Service from 1934 to 1946.

After serving 23 years as Professor, he was made Emeritus Professor of Dermatological Research. "Emeritus" did not seem to agree with his energetic disposition however, for a few months later he was reappointed Professor of Research in Dermatology and Mycology.

To his load of teaching undergraduates and graduates in his classes, his coaching of graduate students in investigative work, he added instruction in a much wider field by his discussions in society meetings, the production of a monograph on Xanthoma and 93 papers on a wide range of subjects. His 6th paper reported investigation of a case of dermatophytosis in which he found penicillium brevicaule var. hominis; but his chief interest was in the mycoses invading the internal organs. With H. R. DOUGLAS he reported in 1921, the discovery of blastomycetoid bodies in a sarcoma-like tumor of the leg.

In 1928, STANLEY CHAMBERS and WEIDMAN reported a piece of research that was a forerunner of the discovery of the antibiotic action of penecillin. Their experiments showed that the bacillus subtilis acted to restrain the growth of many fungi. Their opinion was expressed as follows: "The principle underlying this experiment, i.e., the action of associated microorganisms, met with sufficient success to encourage its application to other dermatoses in which the causative microorganism is known and is cultivable."

Soon after the founding of the American Board of Dermatology and Syphilology FRED WEIDMAN was appointed a member and later was made Vice President and Chairman of the Committee on Education. He served for many years, contributing notably to the success of the Board in maintaining the high standard of examination for certification. His presence on the Board also contributed much to lessen the monotony of oral examinations and to increase the pleasure of fellowship among the members.

From 1944 to 1946 he was President of the American Dermatological Association, after having served as its secretary for five years. He also served as president of the Society for Investigative Dermatology in 1944 and was made Honorary Member in 1952. In 1954 he was elected president of the American Academy of Dermatology and Syphilology. He was Past President of the Philadelphia Pathological, Dermatological and Zoological Societies.

The March 1952 number of the Journal of Investigative Dermatology was a Festschrift dedicated to Dr. WEIDMAN with an excellent resumé of his career by Dr. PILLSBURY, from which I have freely borrowed. In FRIEDMAN's History of Dermatology in Philadelphia there is also a fine account of his life.

Long a member of the Mycological Society of America, Dr. WEIDMAN was for years Associate Editor of Mycopathologia and of the Journal of Investigative Dermatology and the Archives of Dermatology and Syphilology. He collaborated in DAVIS' Cyclopedia of Medicine and the Appleton Practitioner's Library.

In 1939 and again in 1943 WEIDMAN was Co-President of the Section on Mycology at the International Congress of Microbiology.

He was also affiliated with several foreign dermatological societies as Corresponding Member of the British Association of Dermatology and Syphilology and of the Societa Dermatologica Sverica and as Honorary member of the Austrian and Cuban Dermatological Societies and the Academia Nacional de Medicina.

Though an indefatigable worker, accomplishing more in his life than most investigators and teachers, one never was given the impression that WEIDMAN was overworked. He seemed to enjoy work and set an outstanding example to the students. He enjoyed teaching and helping others in their attempts at research.

His papers and discussions always enlivened the meetings of our societies. Whenever Fred stood up to speak we knew that we could expect wise, helpful counsel often spiced with humor. He often drew on his knowledge of animal pathology to illustrate the problems in the human.

He was friendly to everyone, patients, students and fellow practitioners. He was fond of practical jokes especially when directed against pomposity.

To my way of thinking FRED WEIDMAN's was an ideal life. He launched himself on a career new to American dermatology and made a great success of it. His advancement was rapid, his capacity for work enormous and his talent for enthusing his pupils and associates great. He did much to raise the standard of American Medicine, he accomplished much valuable research and endeared himself to all with whom he had contact. While doing a great work for his native land, he extended his influence to other lands and benefitted the whole world.

He is greatly missed.

ARTHUR WILLIAM STILLIANS

IN MEMORIAM

MASAO OTA (1885—1945)

MASAO OTA was born 1885 in Ito, Shizuoka-Prefecture, Middle Japan, in an old family of the district. After graduating from University of Tokyo Medical School he entered 1912 its Dermatological Clinic, then directed by Prof. KEIZO DOHI who founded dermatology in Japan. Thus, OTA started his career as dermatologist.

1916 he was appointed professor of South Manchuria Medical College at Mukden. He married 1917 MASAKO KAWAI and later had two sons and three daughters. 1920 he resigned his office at Mukden and next year he went abroad over America to France. In France, mainly in Paris, he studied at St. Louis Hospital under Prof. SABOURAUD and at the Parasitological Institute of University of Paris under Prof. BRUMPT. 1924 he returned home and was appointed professor of Aichi Medical College at Nagoya. 1926 he was transferred to Sendai and appointed professor of University of Tohoku. 1937 he was called to the post of director of the Dermatological Clinic of University of Tokyo from which he first started twenty five years ago as dermatologist. There he served until he died 1945.

As to OTA's achievements in mycology, he first published 1918 an article in which he presumed so-called Pompholyx as dermatophytosis (Jap. J. Dermat. **18**, 1, 1918; **19**, 788, 1919), and 1921 another article on Trichophytia in Manchuria (Ibid. **21**, 201, 330, 1921). He named a new species of *Microsporon* obtained there *Microsporon ferrugineum* OTA which was later renamed as *Grubyella ferruginea* OTA ET LANGERON and published in Bull. soc. path. exot. **15**, 588, 1922 and Ann. parasit. hum. Comp. **1**, 305, 1923. Up to 1923 he wrote three more articles on Cultivation of *Microsporon furfur* (Jap. J. Dermat. **19**, 875, 935, 1919), on Eczema marginatum in

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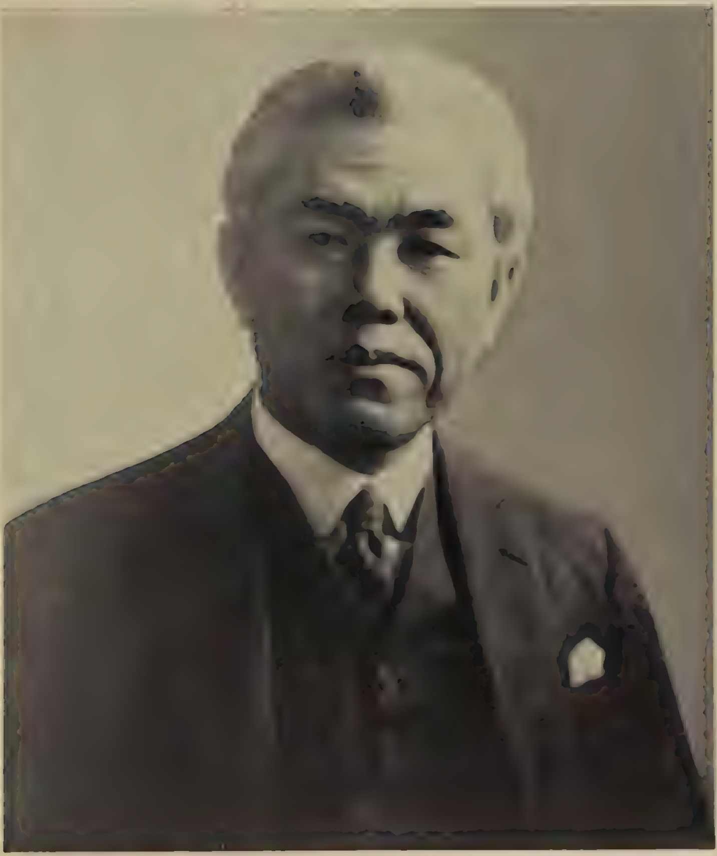
EDITOR's Note. — The psychological climate following the second World War and the torn international connections did not permit the publication of an earlier obituary on Prof. OTA. His colorful personality was well known in Europe, particularly in France and in Germany and, thus, it seems only just that the Western World should take notice, even though belatedly, of the demise of this noted mycologist, a leader of Japanese Mycology. Among his mycological investigations the discovery of *Microsporum ferrugineum* OTA was, from the standpoint of geographical pathology, the most valuable which stood the test of time. While we have two species of *Microsporum* fungi, *Audouini* and *lanosum*, as causative agents of tinea microsporina, in East Asia tinea microsporina is caused exclusively by *M. ferrugineum* OTA. This Editor was able to verify this fact, while on Okinawa during the second World War. Tinea microsporina was endemic there among the children. Its clinical picture and the microscopic features of the infected hairs were exactly the same, as we know it in the Western World. However, the cultures yielded constantly *M. ferrugineum*. — To the obituary from the pen of Prof. KITAMURA, Doctor JUNICHI YASUDA prepared the bibliographic note.



MASAO OTA
(1885—1945)

Japan (In collaboration with KAWASAKI, K. Ibid. **22**, 758, 1922) and on *Trichophyton purpureum* BANG and two other Trichophyton species (Ibid. **23**, 189, 1923). The first one was presented to University of Tokyo Medical School and thereby he won a degree of M. D. In Paris, at the Parasitological Institute of Prof. BRUMPT he carried out with Dr. LANGERON many investigations. Nouvelle classification de dermatophytes (Ann. parasit. **1**, 305, 1923) is one of the products of their collaboration. His idea of classification of pathogenic fungi founded on his own observations was arranged in a very comprehensive article: Champignons parasites de l'homme. Etudes morphologiques et systematiques, which was published serially in Jap. Dermat. **26**, 111, 751, 1926; **27**, 152, 909, 1927; **28**, 381, 1928. Possessing a large collection of fungi, sent to him also from abroad and keeping connection with world mycologists, he had been for many years a central figure of mycological research in Japan. It had been a custome that any fungus strain, so far as newly discovered, was sent to him for identification.

In the field of dermatology he devoted himself to many-sided studies of various skin diseases such as Toxicodermas, Diseases of Sweat and Sebaceous glands, Skin tumors, Nevi and Phacomatoses, Diseases due to Animals, Endocrinological Disorders and Skin Diseases etc. In 1938 he first described a kind of pigmented nevus often seen among Japanese women as *Nevus fuscoceruleus ophthalmomaxillaris* OTA (TANINO, H.: Jap. J. Dermat. **46**, 435, 1939; **47**, 181, 1940). Leprosy also was for him one of the most attractive subjects of study. His new method of Intramuscular Inoculation of Chickens with Human Leprosy was published in Zikken Igaku Zasshi, **24**, 1920, 1940.

OTA, very virtuous by nature, very proficient in German and French, and richly endowed with talents for literature, had been famous from his youth as a poet. He also wrote many novels and dramas. For such literary works he used his pen-name: MOKUTARO KINOSHITA. Furthermore, his travelogues covering wide areas of the Orient and Europe, historical studies on the influx of Christianity into mediaeval Japan and fine art critiques are all highly estimated. He himself was also skil ful in painting and was a lover of flowers.

OTA, a most distinguished scholar of his time and a man of rare versatile talents, died of cancer of the stomach in Tokyo October 15. 1945.

KANEHIKO KITAMURA, Tokyo

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EDITORIAL

With this issue *Mycopathologia et Mycologia Applicata* is introducing a new feature, a *Gallery of Contemporary Noted Mycologists.*

Scattered all over the world, members of the international community of mycologists rarely have the opportunity to meet each other and to have a personal impression of one another. In the feature of a likeness there are traits which reveal the inner man and give us a better idea of one another than writing alone can do. This, in turn, should bring workers in the same field closer to each other. Moreover, a deep historical sense prompts us to help preserve the likeness and memory of those who contributed so much to the progress of mycology in this century. This will also make the work of the historian easier in 2057 by being able to take a pictorial revue of those who made mycological history in the previous century.

TIBOR BENEDEK.

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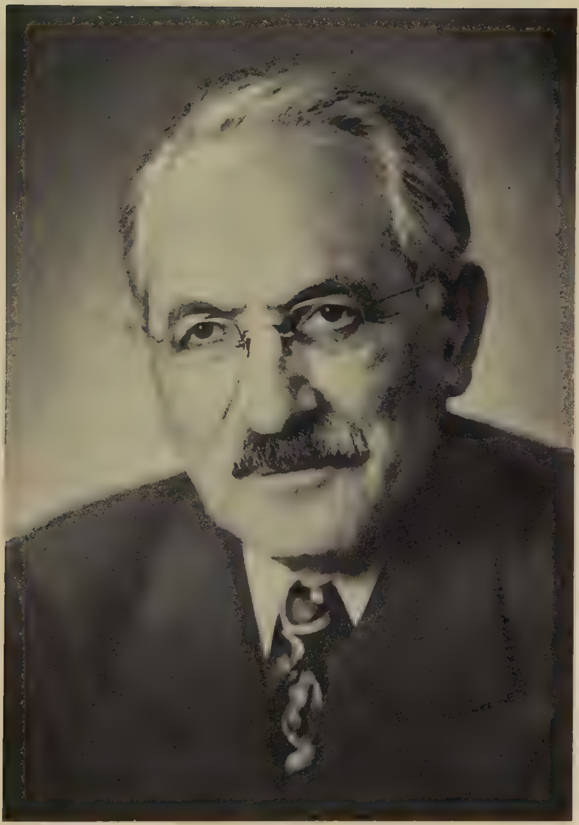

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REVISIONE DI ALCUNI *CEPHALOSPORIUM* ISOLATI DA CASI PATOLOGICI UMANI [1]

R. Ciferri, P. Redaelli (+) e A. Giordano

(*Centro di Micologia Medica, Sezione Botanica presso l'Università di Pavia e Sezione Medica presso l'Università di Milano; Istituto di Anatomia ed Istologia Patologica dell'Università di Milano.*)

(28.III.1957)

(Con 3 figure)

1) *Cryptomyces pleomorpha* Gruner

Il fungo in questione è stato isolato nel Canadà e pubblicato da Gruner (1935) dal sangue di un paziente affetto da carcinoma del polmone con metastasi intratoraciche, operato nel 1930. Dobbiamo alla cortesia del Dr. O. C. Gruner, del Dipartimento di Chirurgia dell'Università McGill di Montreal, l'aver potuto studiare personalmente una coltura di questa.

Il microrganismo, che sarebbe stato osservato anche in sezioni del granuloma escisso e, precedentemente, in casi consimili, è stato studiato e figurato con cura, per ciò che si riferisce alle caratteristiche culturali e biochimiche; meno completo e, sopratutto, assai più confuso è lo studio della micromorfologia, il che ha avuto per conseguenza un'erronea classificazione della specie. L'Autore avrebbe trovato delle artrospore del tipo *Schizosaccharomyces*; delle blastospore come in *Torulopsis* o in *Saccharomyces*; delle ascospore come in *Zygosaccharomyces*; delle minutissime microspore; delle clamidospore; delle ,,gemme laterali'' come in *Sporotrichum*; degli sporangi sessili come in *Mucor*, e forse una fase ultrafiltrante, oltrechè del ben sviluppato micelio. La segnalazione di una così eccezionale morfologia giustificava il nostro interessamento alla specie in questione, che, da un primo sommario esame, appariva piuttosto come una Moniliacea a conidi capitolati.

Già all'esame del materiale di cultura, la specie si rivelava come una tipica specie del genere *Cephalosporium*, di facile cultura ed a rapida fruttificazione [2]. La colonia gigante, in agar glucosato, dopo 15 giorni d'incubazione a temperatura ambiente (15—18°C), è notevolmente sviluppata, superficiale, dapprima nivea, filamentoso-raggiata, quindi di color bianco-avorio, ed infine bianco-sporca, opaca, con una porzione centrale emergente dal substrato, formante una cresta cordonata continua con delle rare formazioni coremioidi aeree. Da queste porzioni si dipartono dei fasci d'ife bianche, perpendicolari, disposti come le barbe di una piuma, che verso la periferia sono più rare e distanziate, e quindi scisse nelle ife miceliari, cosicchè il margine della colonia apparisce molto finemente piumoso. Il rovescio della cultura è bianco-giallastro. In cultura sullo stesso terreno, di 10 a 15 giorni d'età in capsule Petri si ha un'abbondantissima fruttificazione con formazione di capitoli acrogeni e mucillaginosi, di conidi portati da conidiofori di lunghezza variante da pochi sino a 40 μ. I capolini sono, generalmente, perfettamente sferici, piccoli (del diametro medio di 7—10 sino a 12 μ). Lungo il micelio si vedono frequanti clamidospore di forma varia, più spesso allungate ed a parete spessa. Ogni capitolo include un numero variabile di conidi bastonciniformi, ad apici nettamente troncati, diritti o leggermente incurvati, di 4,5—6 × 1,8—2 μ, frammisti a più rari conidi minori dei precedenti, quasi batteroidali, in media di 2,5—3 × 1,5 μ. In goccia pendente in acqua pepto-glucosata, dopo 15 giorni di cultura a temperatura ambiente, si ha un discreto sviluppo del micelio, formato da ife sottili (2—2,5 μ di diam.), settate, irregolarmente ramose talvolta anastomosate. Le fruttificazioni sono rare; i conidiofori, non ramosi e non settati, diritti (o più raramente, quasi sinuosi) sono lunghi in media 18—20 μ. (più raramente sino a 30 μ), di circa 2,5 μ di diametro, sono inseriti irregolarmente ma ad angolo retto sulle ife miceliche. I capitoli del diametro di 7—10 μ si risolvono nei conidi che sono bastonciniformi, di 4,5—6 × 2 μ, e pochissimo numerosi. Nelle nostre culture non abbiano mai osservato forme artrosporiche e forme blastosporiche.

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[1] Queste note furono redatte in gran parte tra il 1938 e il 1939; poi, per varie ragioni, rimasero inedite. Le ripubblichiamo con dati bibliografici aggiornati ed un maggiore materiale critico, anche in omaggio al comune amico, il compianto Prof. P. Redaelli.

[2] Era presente un batterio dal quale la cultura fu purificata prima dello studio.

Trattasi dunque di una specie del genere *Cephalosporium* Corda, quale è tradizionalmente considerato, e quale tipificato dal *C. acremonium* Corda, che ben poco differisce da tutte quelle note nel campo della patologia umana e studiate comparativamente dalla PINKERTON (1936) poi, indipendentemente, da BARBOSA (1941) come *Hyalopus*.

La sola differenza rispetto alla descrizione è l'assenza di forme gemmanti e scissipare, che il GRUNER indica e che sarebbero scomparse, se mai sono state presenti.

La nostra impressione è che, in realtà, le presunte forme-lievito non siano state altro che conidi, che si formano precocemente in culture giovani da conidiofori molto corti o quasi nulli, e che si isolano dai capitoli accollandosi lungo le pareti delle ife miceliche, anche per la sola coesione dei films liquidi. Tali conidi così irregolarmente aggregati possono dare l'impressione di forme-lievito prodotte dalle ife. Nelle culture pienamente sviluppate, ove conidiofori e capitoli di conidi sono normali, la morfologia ritorna ad apparire pure normale.

Poichè l'unico carattere sostanziale che fa differire il *C. pseudofermentum* dal *C. acremonium* è la presenza di questa forma-lievito, riteniamo, sino a prova contraria, che il *Cryptomyces pleomorpha* sia da identificare con il *Cephalosporium acremonium*.

Deve notarsi che manca una diagnosi del genere *Cryptomyces* e del *C. pleomorpha*, e una giustificazione della posizione sistematica, che viene detto situarsi tra gli Ascomiceti.

Ciò a parte, il nome generico *Cryptomyces* è stato impiegato in tutt'altro senso ed è sempre valido.

GREVILLE (1826), infatti, descrisse, tra gli Ascomiceti Facidiali, un genere *Cryptomyces*, di cui almeno una delle due specie, *C. maximus* (Fr.) Rehm (1888) è ben nota, per provocare una malattia del Salice. Tra i lavori relativamente più recenti si veda ALCOCK (1926).

Le prove di patogenicità per inoculazione di patine culturali in Ringer via endoperitoneale e sottocutanee a cavie e ratti furono negative.

2) Due ceppi umani patogeni di *C. acremonium*

Nel 1938 il Prof. B. BESTA, dell'Istituto Forlanini di Roma, ci inviò per lo studio due ceppi di *Cephalosporium* indifferenziabili dal *C. acremonium* Corda, dal punto di vista culturale e morfologico.

Uno (Ceppo B. 28) fu isolato da una semina di sangue (metodo Löwenstein) su terreno Petragnani normale, dal cuore del cadavere di un tal B. D. affetto da t.b.c. cronica del lobo superiore destro e disseminazione miliare con una caverna isolata e recente al lobo superiore sinistro e sinfisi pleurica.

L'altro (Ceppo B. 814) fu isolato dal sangue circolante di una paziente M. F., febbrile, mostrante piccoli focolai apicali inattivi, pacchi ghiandolari di discreto volume alle stazioni linfatiche. Il fungo è stato isolato egualmente su terreno Petragnani.

Le prove di patogenicità e immunologiche eseguite dal Prof. Besta, dimostrarono che il ceppo B. 28 per inoculazione endovena a due conigli fu negativa. Inoculato endoperitonealmente a quattro cavie, due sacrificate dopo nove settimane, mostravano fegato e milza degenerati con scarsi ascessolini evidenti al taglio. Nulla agli altri organi. In due ratti inoculati endoperitonealmente e in due endotesticolarmente non si ebbero reazioni.

Analoghe inoculazioni si eseguirono col ceppo B. 814, gli animali essendo sacrificati circa 7 mesi dopo le inoculazioni. Negli animali si osserva iperplasia di tutto il tessuto linfatico. Nei polmoni reazione iperplasica delle cellule linfatiche normalmente esistente e modesta proliferazione degli elementi istiocitari e particolarmente dei setti. In alcuni animali i fatti suddescritti sono più spiccati e si giunge alla formazione di veri e propri noduli granulomatosi, talvolta con atelettasia delle parti polmonari finitime.

Con la colorazione allo Stropeni si osserva, oltre l'iperplasia di elementi linfatici, la formazione di veri e propri noduli granulomatosi, per lo più costituiti da cellule istiocitarie in varie fasi di evoluzione, con scarsa reazione ematica e notevole partecipazione linfoide.

Sono presenti plasmacellule. Nelle sezioni si vedono scarse formazione ifiche, talvolta anche di forma clavata.

Con micetina analoga, il ceppo B. 28 diede una intradermoreazione negativa con reazione febbrile dopo 48 ore, su conigli e su cavie. Con micetina eterologa analoga (dal ceppo B. 814) intradermoreazione pure negativa, a reazione febbrile quasi nulla. Tubercolinoreazione negativa.

Potere anafilattogeno su due cavie reinoculate endovena con lo stesso ceppo dopo circa 4 mesi: polipnea, tremore intenso, con scosse o contrazioni. Vi è reazione febbrile: dopo 30 minuti la temperatura è 39,5°C. Questa sintomatologia si accentua e l'animale arruffa il pelo. Dopo circa 20 primi la sintomatologia è scomparsa. Il controllo dà solo una modica polipnea, subito cessata.

Con il ceppo B. 814 sono state eseguite le stesse prove con risultati identici a quelli ottenuti sul ceppo B. 28. La prova anafilattogena con micetina eterologa analoga (dal ceppo B. 28) ha causato solo una modesta polipnea senza tremore, ecc. e una lieve caduta di febbre.

3) Tre ceppi di *Cephalosporium* subg. *Allantospora*

I risultati concernenti questi tre ceppi vengono esposti assieme, in vista della loro identità.

Uno è l'*Allantospora violacea* Ambrosioni (1938) isolata da un caso di erosione interdigitale e plantare del piede in Italia, il cui ceppo fu ottenuto direttamente dallo scopritore (A. V.).

L'altra è l'*Allantospora onychophila* Vuillemin (1932), nel ceppo che l'ASCHIERI (1932) ridescrisse come *Hyalopus onychophilus* (Vuill.) Aschieri (A. O.).

Il terzo è un *Cephalosporium* subg. *Allantospora* isolato da uno di noi (CIFERRI) dallo sputo di un t.b.c. proveniente dalla Clinica Medica dell'Università di Pavia, sul quale, già al tempo dello studio, non avemmo dettagli del caso clinico (A. C.).

Caratteristiche culturali su agar glucosato a temperatura ambiente (20°C circa) (A. V.) — Dopo quattro giorni scarso sviluppo di una vegetazione verde-giallastra, con sfumature qua e là verdognole a bluastre, sulla quale si elevano numerosi ciuffetti bianchi di una vegetazione aerea micelica. Dopo nove giorni le ife reptanti si estendono occupando gran parte della superficie dell'agar, mentre la vegetazione aerea, pur rimanendo soffice, si ispessisce. Il colore bianco-verdastro della colonia si accentua. Dopo 18 giorni la vegetazione superficiale è di color bianco-sporco, que a là chiazzata di color brunastro chiaro. Il micelio sterile aereo ricopre la sottostante parte fruttificata della colonia da cotonosa a ragnatelosa, che assume un colore tabacino sino ad umbrino, qua e là persino bruno-marrone scuro con efflorescenze ocracee. Più tardi ancora la parte fertile appare polverulenta, con una patina alabastrina, giallastra o sporca, een margini finemente frangiati, più o meno regolarmente festonati sino a lisci.

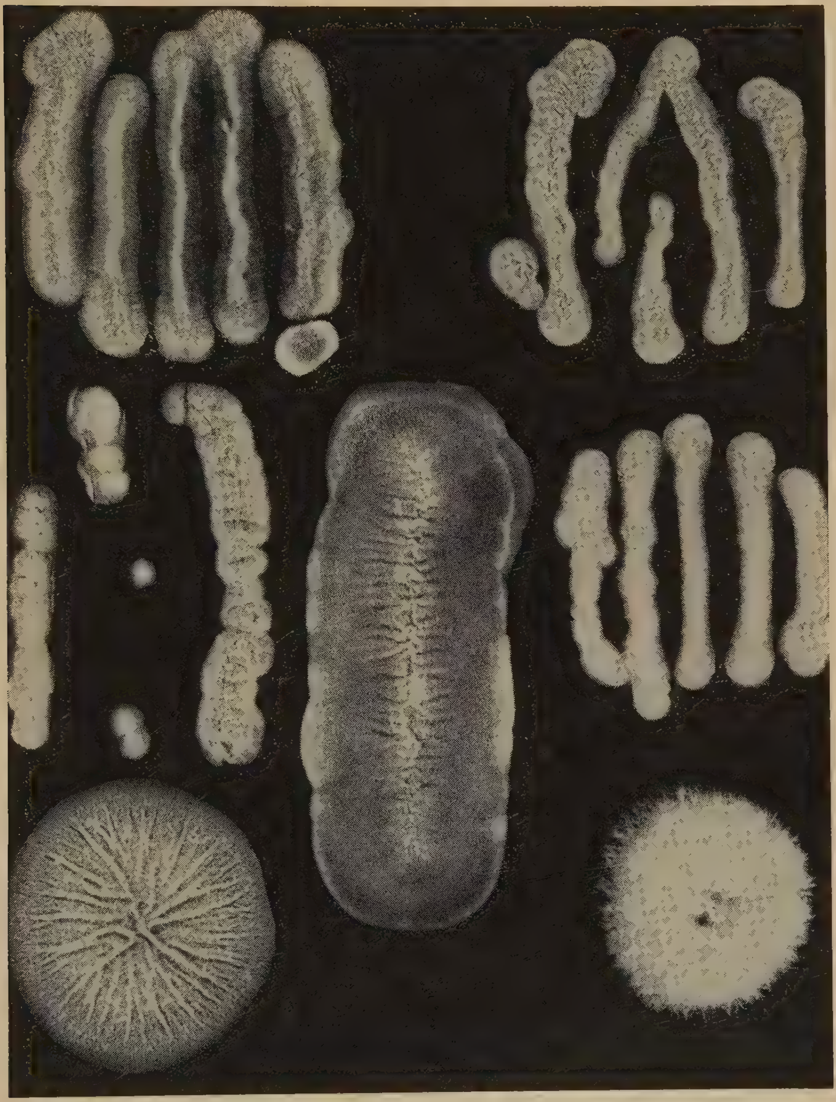

Tavola I. Colonie di *Cephalosporium* spp. Su Agar Sabouraud

In alto a sinistra: *C. acremonium* Cda. (ceppo Besta C. 28). — In alto a destra: Idem (ceppo Besta B. 814). — In mezzo a sinistra: *C. onychophilum* (Vuill.) Coudert (ceppo *Allantospora violacea* Ambr.). — In mezzo nel centro: *C. acremonium* Cda. (ceppo *C. stuhmeri* Schm. et v. Beyma). — In mezzo a destra: *C. acremonium* Cda. (ceppo *Acremonium kiliense* Gruetz). — In basso a sinistra: *C. acremonium* Cda. (ceppo *Cryptomyces leomorpha* Gruner). — In basso a destra: *C. acremonium* Cda. f. coremioide (ceppo *C. spinosum* Negr.).

Colonie di 7 giorni circa d'età, con eccezione di quella centrale del mezzo di 15 giorni, e delle due in basso di circa 30 giorni.

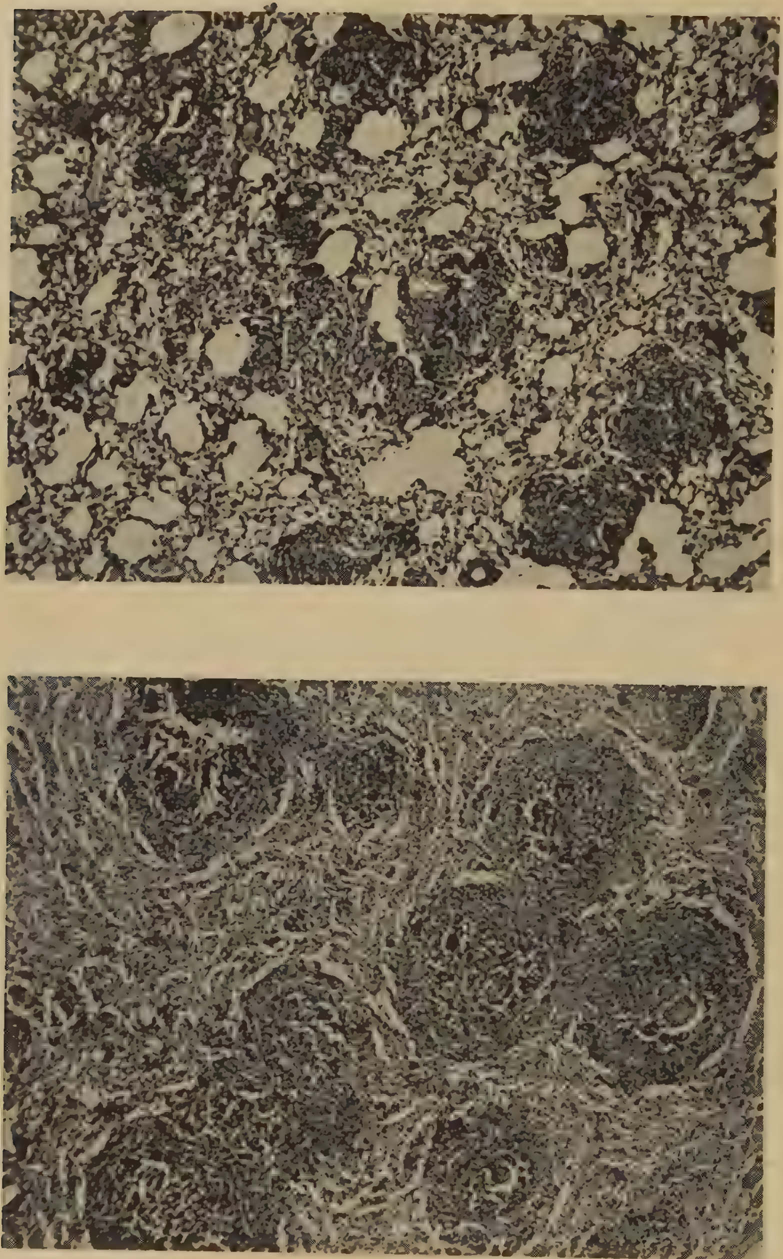

Tavola II. Sezioni di organi di cavie inoculati con *C. acremonium* Cda.
In alto: polmone di animale inoculato con il ceppo B. 814 Besta (70 diam.).
In basso: milza di animale inoculato con il ceppo B. 28 Besta (50 diam.).

(A. O.) Dopo quattro giorni sviluppo e aspetto simile al ceppo A. V. ma con vegetazione aerea di più limitato sviluppo e grigiastra, mentre tutta la colonia è finemente rugosa e vellutata, pellicolosa e raggiata alla periferia. Il colore dominante è una sorta di grigio-giallastro. Dopo 9 giorni l'aspetto non è molto modificato; sempre scarsa è la vegetazione aerea, mentre più densa è quella di fondo, che assume un colore giallo-limone sporco. Dopo 18 giorni l'aspetto è analogo a quello del ceppo A. V., ma il colore della colonia è isabellino, qua e là vagamente purpurascente. Solo il micelio aereo rimane sempre molto scarso.

(A. C.) Dopo 4 giorni sviluppo rapido, secondo una patina a trasparenza alabastrina, di colore grigio-giallastra, opaca, raggiata alla periferia; quindi giallastra con sfumature di color rosa antico. Vi è un accenno di assolcature alla periferia, mentre il micelio aereo è sempre scarso, ed appena esteso sopra una patina vellutata di fondo. La vegetazione aerea bianca è meglio manifesta verso il nono giorno, mentre la colorazione generale volge al rosa con sfumature color salmone. Dopo 18 giorni la vegetazione sterile aerea è tuttora scarsa, quasi assente; la colorazione è uniforme ed opaca, con accenni a strie longitudinali e trasversali. Vi sono rari cordoni miceliari suberetti tipo coremi. Il colore dominante è sempre il rosa antico o il rosa fior di pesco, con sfumature salmone sino ad ocracee.

In brodo glucosato, a temperatura ambiente. (A. V.) Dopo 4 giorni, si ha un micelio a ciuffo superficiale, mammellonato, biancastro con sfumature grigiastre, verdicce o bluastre. Il micelio chiaro tende a risalire sui bordi del vetro. Dopo 9 giorni la colonia è giallastra con chiazze verdognole.

(A. O.) Dopo 2—4 giorni si ha una membrana compatta, glabra, giallastra, con scarsa vegetazione aerea; micelio c.s. Dopo 9 giorni la colonia è chiazzata di color giallo-verdastro.

(A. C.) Dopo 2—4 giorni vegetazione più scarsa, che da un anello periferico tende a formare una colonietta bianco-giallastra sporca, con scarse efflorescenze bianche; micelio c.s. Dopo 9 giorni tutta la colonia tende al color rosato.

In culture più vecchie i tre ceppi di *Allantospora* formano un pigmento brunastro che si diffonde nel liquido di cultura, più abbondantemente sul ceppo A. V., il cui brodo diviene di color bruno-rugginoso; meno manifestamente negli altri due ceppi, ove il colore del liquido è rosso-brunastro chiaro.

Caratteristiche microscopiche — Il ceppo A. V. ripete le caratteristiche microscopiche così ben descritte dall'AMBROSIONI. Vi è però una certa tendenza verso una regressione morfologica nel senso che i conidiofori descritti come frequentemente ramosi, tendono ad essere semplici, ed i conidi continui prevalgono numericamente su quelli 1—2-settati, generalmente di 3—4 × 5—7,5 μ, raramente sorpassando i 10 μ di lunghezza. I conidi falcati fusariformi, a 3—7 setti trasversali, sono del tutto eccezionali. Vi è pure tendenza ad una riduzione nel numero dei conidi che costituiscono il capitolo.

Clamidospore miceliari intervallari numerose e varie, di solito isolate talvolta 2—3 catenulate.

Ciò significa che la morfologia del ceppo A. V. si avvicina sensibilmente a quella del ceppo A. O. secondo la descrizione dell'Aschieri, e che si mantiene nelle nostre culture. Ivi i conidi 1—2-settati sono rari o rarissimi, ed allora possono sorpassare i 10 μ di lunghezza (pur senza raggiungere mai i 18—20 μ indicati dall'A.). I conidi sono spesso più stretti (2,5—3,5 μ di larghezza) ma di eguale lunghezza media (6—8). Capolini assai variabili.

Le clamidospore sono del tipo di quelle del ceppo A. O. Il ceppo A. C. ripete le caratteristiche del ceppo A. O., ma la presenza di conidi falcati e settati trasversalmente è assolutamente eccezionale. Anche le clamidospore sono piuttosto scarse.

Secondo la nostra opinione, i tre ceppi sono riferibili ad un'unica specie che, non accettando i generi *Allantospora* e *Hyalopus* (vedi, in merito, oltre ai citati PINKERTON e BARBOSA, anche RICHON, 1892 e BUCHANAN, 1911) sono da comprendere sotto il binomio di *Cephalosporium onychophilum* (Vuill.) Coudert [1]).

4) *Acremonium kiliense* Gruetz

Di questa specie si sono studiati due ceppi: uno, trapianto dell'originale descritto da GRUETZ nel 1925, ma che ha circolato sotto il binomio *Cephalosporium kiliense* (Gruetz) Hartmann (1926). Un secondo ceppo, etichettato come *C. asteroides* [2]) ed isolato da uno di noi (REDAELLI) con CABRINI da un caso di presunta micosi tonsillare a Pavia [3]).

Il *C. kiliense* (Gruetz) Hartmann è stato oggetto di parecchie controversie. Descritto dal GRUETZ (1925) come *C. kiliense*, modificato il binomio in quadrimonio da BENEDEK (1928), lo stesso GRUETZ (1928) riferì il ceppo a *C. acremonium*, in ciò confermato in una delle schede e dei preparati da POLLACCI e NANNIZZI (1930) (e quindi da KROEMER, 1936), in polemica con BENEDEK (1929).

Il ceppo da noi studiato — lo stesso già pubblicato de POLLACCI e NANNIZZI, e trapianto dell'originale di GRUETZ — non sembra potersi differenziare dal *C. acremonium*. Nelle nostre culture il ceppo non è mai stato grigiastro, ma talvolta — e tardivamente — roseo. Le circonvoluzioni delle colonie talvolta sono più ampie di quelle di un tipico ceppo di *C. acremonium*, ove già, però, queste caratteristiche sono variabili. In mezzo liquido zuccherato si ha rapidamente un anello quasi completo, e dopo 4 giorni una vegetazione superficiale completa, con vegetazione aerea scarsa o nulla. Al microscopio i caratteri sono gli stessi della specie suddetta.

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[1]) COUDERT (Guide Prat. Mycol. Médic., pag. 315. 1955) riporta questa specie come *Cephalosporium* e, per quanto ci consta, la combinazione dovrebbe essere attribuita a questo studioso.

[2]) Probabilmente sulla base della nomenclatura quadrinomiale di BENEDEK (1928): *C. asteroides griseum gruetzii.*

[3]) Questo caso é ricordato da NANNIZZI (1934, p. 454).

Anche il secondo ceppo è un tipico *C. acremonium* (dei quali è inutile ripertere qui le caratteristiche), come, del resto, per le specie di Gruetz, era stato supposto da BENEDEK (1929).

Come gli altri *Cephalosporium* del tipo *acremonium*, i mezzi liquidi non sono pigmentati o lo sono appena in colore rosato.

5) *Cephalosporium sérrae* Maffei

Questa specie isolata da SERRA (1929) da un caso di cheratomicosi dell'occhio, è stata descritta dal MAFFEI (1929).

Questo micete è stato causa di una discussione in merito alla sua possibile identità con il *C. stuehmeri* (vedi al no. 8 della presente contribuzione), ma indubbiamente le due specie sono distinte tra di loro.

Ben poco abbiamo da aggiungere alla buona descrizione che ne diede il MAFFEI. Le colonie su mezzo solido glucosato ricordano molto quelle dei *Cephalosporium* subg. *Allantospora* citati in precedenza, per il colore che passa attraverso varie sfumature, dal rosato sino al giallo-rossastro, poi al rosso-bruno ed infine, qua e là, al bruno. E' notevole la scarsezza di micelio aereo.

Le particolarità più interessanti si hanno nei conidiofori, che di solito sono ramosi, quasi verticillati, riuniti in gruppi di 2—6, di solito 3—4-ramosi. Ancorchè generalmente i capitoli sieno formati allo stesso livello o quasi, talvolta i rami secondari sono di lunghezza dissimile, e, se raccorciati, l'aspetto è verticillioide. Non mancano però conidiofori semplici (non ràmosi) e persino molto brevi e tozzi. I conidi non sono settati e lunghi 8 μ, sino 10—12 μ; talvolta hanno un setto trasversale centrale. Numerose le clamidospore intercalari, molto varie per l'aspetto.

E' questa una specie ben distinta da tutte le altre isolate dall' uomo per la ramificazione, spesso verticillata, dei conidiofori.

Secondo la nostra opinione, però, questa specie è indistinguibile dal *C. subverticillatum* Schultz et Saccardo nella cultura che ne avemmo da VERONA e che questi pubblicò nel 1932. La specie fu poi studiata dal punto di vista biochimico del LUCHETTI (1934), essendo interessante anche perchè isolata da petrolio. I conidiofori sono bifidi o trifidi, ed anche inequilateri, con conidi che vengono indicati di 6—8 × 4 μ, ma che nei nostri trapianti erano in media dell'ordine di grandezza di quelli del *C. serrae*.

VAN BEYMA (1940) ha riportato queste specie al genere *Verticillium* con *V. serrae* (Maffei) v. Beyma, ciò che a noi non sembra giusto Egli ha dovuto studiare il ceppo a fruttificazioni depauperate da vari anni di cultura, e quindi del tipo verticillioide.

6) *Cephalosporium spinosum* Negroni

Il ceppo originale di questa specie, descritta nell'Argentina da NEGRONI (1933) da un caso di onicomicosi, ci fu inviato dal NEGRONI stesso, ma non si poté studiare completamente.

Il tipo di colonia è quello dei *Cephalosporium* subg. *Allantospora* precedentemente descritto, con sfumature che vanno dal rosa-sporco al porpora-rossiccio ed al rosse-brunastro. Ma la superficie delle colonie è membranosa ed irsuta per la formazione di coremi di ife sterili, altri sino a 0,5—1 mm (eccezionalmente di più), irregolarmente sparsi, talvolta quasi spinescenti. Inoltre lo sviluppo delle colonie è lento.

In mezzi liquidi zuccherati si ha un pigmento rosso-bruniccio che si diffonde nel mezzo.

La morfologia microscopica è quella di un *Cephalosporium* a conidi del tipo *C. acremonium* e conidiofori semplici, ma anche con conidi falcati, 1—3-eccezionalmente 4-settati, anchedi 12—15 × 3,5—4 μ. Si hanno clamidospore.

Barbosa (1941) dà i coremi come fertili, ciò che farebbe del fungo una Stilbacea. Il già citato Barbosa distingue queste specie dal suo *C. cordoniformis* perchè ivi, oltre ai coremi, si hanno degli aggregati cordoniformi di ife, e perchè non produce pigmento diffusibile nel mezzo; ma tutto lascia credere che questa specie sia molto vicina — e forse solo una varietà o forma — del *C. spinosum*, che — a sua volta — ha forme di transizione verso il tipico *C. acremonium*.

7) *Cephalosporium rubrobrunneum* Benedek

L'origine di questo ceppo non è chiara. Forse è un trapianto del ceppo di Benedek che ha circolato appunto come *C. rubrobrunneum* Benedek.

Le colonie, nelle nostre culture, non sono però mai state bruno-rosse, ma tra il rossastro e il rosa, il pigmento sviluppandosi tardivamente.

La morfologia microscopica è quella consueta del *C. acremonium*.

8) *Cephalosporium stuhmeri* Schmidt et v. Beyma

E' un trapianto del ceppo originale, descritto da Van Beyma (1933) e che Focosi (1934) volle, erroneamente, identificare col *C. serrae* Maffei, ciò che negò lo stesso van Beyma (1935).

Secondo le nostre ricerche, trattasi ancora di un *C. acremonium*, a colonie descritte come più profondamente solcato-radiate che d'uso e di colore rosso-bruno chiaro, ma che nelle nostre culture non era differenziabile da una qualsiasi colonia di *C. acremonium*: forse appena più rosee come colore e più tinte al rovescio.

La morfologia microscopica è ancora quella del *C. acremonium*, con conidiofori semplici ed eretti, e conidi non diversi da quelli di questa specie.

Conclusioni

La sistematica dei *Cephalosporium*, anche limitatamente alle specie isolate dall'uomo, è in uno stato caotico, che già comincia dal significato e la validità dei vari generi che convergono in *Cephalosporium*, e prosegue nella discriminazione e l'individuazione delle specie.

Ciò è evidente anche nella monografia della PINKERTON (1936) e di BARBOSA (1941) del resto già divergenti tra di loro.

Questa situazione è stata ben delineata nella rassegna critica comparativa francese di COURTELEN, COCHET e BIGUET (1948) a proposito di un ceppo di *C. acremonium.*

Volendo riassumere i risultati delle nostre osservazioni, dei ceppi studiati paiono separarsi tre specie:

1) il tipo *C. acremonium* Corda, secondo i ceppi che sotto questo binomio si isolanò e circolano nei laboratori;
2) il *C. serrae* Maffei a conidiofori ramoso-verticillati, che consideriamo eguale al *C. subverticillatum* Schulz et Sacc. poichè l'assegnazione a *Tilachlidium* per parte di VUILLEMIN (1912) non pare sostenibile. La sistematica di questo genere sarebbe da rivedere rispetto al genere *Acrostalagmus*, se considerato come valido in relazione ai conidiofori ramosi (vedi PETCH, 1925).
3) il *C. pseudofermentatum* Ciferri che ha una ben manifesta forma-lievito gemmante se pur non si tratti di un'associazione del *C. acremonium* con un lievito, come su altro ceppo avrebbe osservato la PINKERTON già citata (ma il CIFERRI non riuscì ad isolare l'eventuale lievito dalla forma conidica ed ifica);
4) una specie di *Cephalosporium* a conidi anche falcati e trasversalmente 1-plurisettati, e di cui forma il binomio più vecchio è *C. onychophilum* (Vuill.) Coudert. La posizione sistematica di questa forma sarebbe da rivedere in relazione al genere *Cephalothecium.* A questa specie — o gruppo di specie — pare doversi riferire il subg. *Ciferriella* Barbosa (1941) del gen. *Cephalosporium.*

Salvo il *C. nigrum* Kambayashi (1937) che avrebbe le colonie nere — e che potrebbe non essere affatto un *Cephalosporium* — tutte le altre specie riconoscibili della descrizione paiono riportarsi ad una delle specie suddette, sopratutto al *C. acremonium.* Stando alle osservazioni di LUCHETTI (1939) la formazione di coremi sarebbe una caratteristica di ceppo nell'ambito delle specie: dunque, al massimo, di una varietà o forma.

Dati più sicuri potrebbero aversi se fossero noti i rapporti con forme perfette. In realtà, stando ai dati della letteratura, i *Cephalosporium* fanno parte del ciclo di vari Ascomiceti, da *Allescheria* (per *A. boydii*), a *Ceratostomella* (per *C. picea*); da *Cordiceps* (per *C. militaris*) ad *Emericellopsis* v. Beyma (= *Saturnomyces* Cain), al dubbio genere *Plectosphaerella*; alla più recente attribuzione a *Ophiostoma* (per *O. persicinum*). Più incerti sono i rapporti con *Gibberella* e, comunque, le relazioni con *Fusarium*, più volte asserite e più volte negate.

Incerte paiono pure le relazioni con i Basidiomiceti Agaricini e il significato biologico di certe specie fungicole come *C. costantini* e *C. lamellaecola.*

Nessun nuovo elemento pare dunque emergere a questo riguardo. Indubbiamente si ha, nel genere *Cephalosporium*, una tendenza, da parte di un gruppo di specie, al parassitismo sugli animali (Crostacei, vari Artropodi, tra cui gli Insetti e i Nematodi), che può eccezionalmente spingersi sino all'uomo.

Del polimorfismo del *C. acremonium* anche come conseguenza di adattamento ai mezzi di cultura, ha scritto il LUCHETTI (1939), inclusa una notevole borofilia, e, in un ceppo, la tendenza a formare coremi, con clamidospore anche assai abbondanti e catenulate. Questo stesso studioso cita pure delle osservazioni di resistenza al l'arsenico.

Summary

Eight species of *Cephalosporium* from man are revised, mostly on original strains. *Cryptomyces pleomorpha* Gruner is a typical *Cephalosporium acremonium*; in addition *Cryptomyces* is a valid Phacidiaceous genus described more than a century ago. Of two human strains of the same species, one has been fully pathogenic for laboratory animals.

Allantospora violacea Ambr., *A. onychophila* Vuill. and an unnamed strain were revised. All are to be referred to *Cephalosporium* (*Allantospora*) *onychophilum* (Vuill.) Coudert.

Two strains of *Acremonium kiliense* are typical *C. acremonium*. *C. serrae* Maffei is a good species, but probably indistinguishable from the saprophytic *C. subverticillatum* Schulz et Sacc., reisolated in Italy in the year 1934.

C. spinosum Negroni — probably indistinguishable from *C. cordoniformis* Barbosa, both South American species — is a coremioidal form of *C. acremonium*.

C. rubrobrunneum (?) Benedek is a simple *C. acremonium*, as well as *C. stuehmeri* Schmidt et van Beyma.

In addition to the previously named species, *C. pseudofermentatum* Cif. (if really possess a yeast-like stage) and *C. nigrum* Kamb. (if this species is pertaining to the genus *Cephalosporium*) are listed.

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TRICHOSPORIUM MANTEGAZZAE POLL. E CLADOSPORIUM CHLAMYDEUM N.SP.

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(4.IV.57)

(Con 3 figure)

Questa nota era stata approntata sin dal lontano 1947. Ancora nell'idea di rendere omaggio al defunto amico e collega Prof. P. Redaelli, ci siamo decisi a darla alle stampe, aggiornandola, per quant'era necessario, in rapporto alla bibliografia e a qualche dato a seguito di più recenti lavori.

I. **Sul** *Trichosporium mantegazzae* **Poll.**

Il *Trichosporium mantegazzae* fu descritto da Pollaci (1922) ed illustrato da due tavole a colori, da una coltura isolata da una forma gommosa e profonda di un paziente affetto da pleuromediastinite infiltrativa diffusa, in cui venne ritrovato più volte, che migliorò notevolmente con una cura iodica e che, inoculato sottocute al topo, produsse delle piaghe gommose dermiche.

Anni or sono avemmo occasione di studiare un trapianto del ceppo originale, poi perduto, e non avemmo difficoltà a reidentificarlo come un tipico *Aureobasidium pullulans* (De By.) Arnaud (= *Dematium pullulans* De Bary; = *Pullularia pullulans* Berkh.; ecc.). Di ciò fan fede, oltretutto, le eccellenti due tavole a colori del Pollacci. Il fungo non fu patogeno o lo fu solo molto debolmente (in borse sottocutanee artificiali diede solo una piaga dermica gommosa).

Su questa specie, oltremodo frequente in natura ed in ogni substrato organico, uno di noi, in collaborazione (Ciferri, Ribaldi e Corte, 1957) ha pubblicato uno studio critico, basato sull,indagine di 23 ceppi, stabilendo una sinonimia di ben 53 sinonimi.

E' dunque un nuovo sinonimo che si aggiunge anche a quelli di funghi medici del ciclo di *Aureobasidium pullulans*, dei quali i più conspicui sono *Cladosporium mansoni* (A. Cast.) Pinoy, *C. werneckii* P. Horta, *Cryptococcus metaniger* A. Cast. e *Hormiscium dermatitidis* Kano.

Clements e Shear (1931) riportano, nelle chiavi analitiche dei funghi, due volte il genere *Aureobasidium* (ivi ribattezzato *Aureobasis*): una volta nelle Hypochnaceae (Agaricales) ed una nei Melanconiales Hyalosporei.

II. Cladosporium chlamydeum n.sp.

Nella nostra micoteca esisteva un secondo ceppo sotto la stesso binomio di *T. mantegazzae* ma isolato nel 1941 dalla cute di un cane affetto da una forma di dermatite eczematosa purulenta, ribelle ai trattamenti con solfo usuali (ma praticamente scomparsa dopo applicazioni iodate in veicolo grasso).

Rivedendo il ceppo originale di Pollacci, fummo indotti a studiare anche questo secondo ceppo, di cui non trovammo nè il nome di chi lo isolò, nè la data, nè altri dati clinici oltre quelli suindicati.

Ancorchè l'aspetto delle culture sui mezzi usuali fosse simile a quelli di certi non tipici ceppi di *A. pullulans*, lo studio delle colonie in via di sviluppo su Sabouraud ci rivelò esservi delle differenze. La morfologia di questa specie in comparazione con quella di *A .pullulans* si osserva bene nelle nostre figure schematiche. Le microfotografie della nostra tavola danno una migliore idea dell'aspetto d'insieme del fungo maturo.

La patogenicità di questa specie per gli animali di laboratorio non risulta essere stata saggiata; ma trattasi probabilmente di un saprofita.

Le colonie su agar Sabouraud e su agar malto sono vellutate, verde-grigie, poi brune-verdicce ed infine nerastre-verdognole, a rapido sviluppo a temperatura ambiente; il rovescio è olivaceo-scuro.

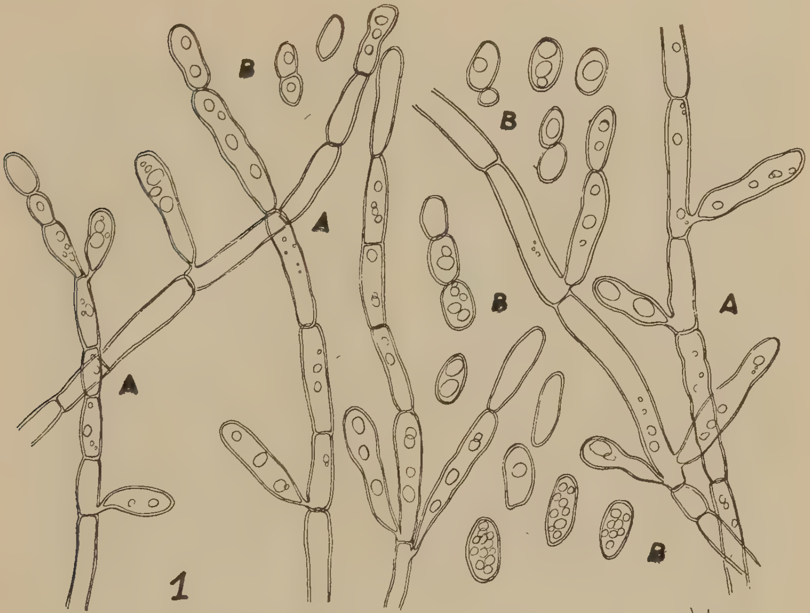
Al miscroscopio il micelio è poco sinuoso e non molto abbondantemente ramificato, composto di ife a segmenti suberetti e non densamente settati, quasi impercettibilmente ristretti ai setti, di diametro molto variabile: 2—3 μ se giovane, sino a 5—6 μ se vecchio. Il colore è fugacemente ialino, poi brunastro ed infine bruno. Le tuniche cellulari sono poco ispessite. Sulle ife sterili si inseriscono lateralmente di solito alternatamente, ma talvolta opposti (ed anche 3—4 verticillati) dei conidiofori tozzi e corti, assai vari come forma e dimensioni, tipicamente cilindrici, ad apice libero arrotondato e alla base molto, ma brevemente assottigliati, non settati, più spesso di 3,5—4,5 μ di diam., dapprima chiari, poi brunastri. La lunghezza arriva a 20—35 μ.

All'apice portano dapprima un solo conidio acrogeno, variabile come forma e per le dimensioni. Tipicamente è ellissoide od ovoide, ma talvolta un poco piriculato o di altra forma, di regola non settato (solo molto eccezionalmente con un setto centrale) chiaro, poi brunastro, a parete non molto sottile, liscia, infine uni-o pluriguttulato. Le dimensioni sono variabili, più spesso di 3—8 × 3—5 μ.

Più spesso il conidioforo rimane monosporo, ma talvolta, mentre il conidioforo si allunga, si può produrre un secondo conidio, poi eventualmente un terzo, formandosi una catenella di non oltre 3 conidi simili, che non ramifica.

Non si hanno forme-lievito per gemmazione di conidi isolati, che germinano emettendo un tubulo miceliare allungato e all'inizio poco ramoso.

Nel substrato agarizzato od alla superficie si vedono delle clamido-



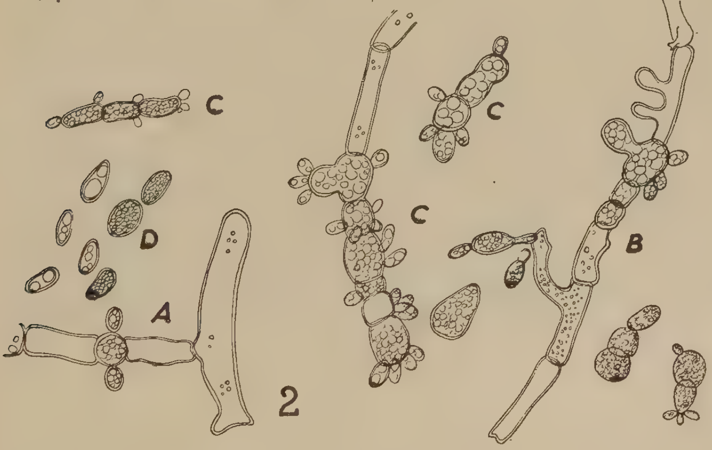


Fig. 1.— *Cladosporium chlamydeum* n.sp.
A) ife fertili con conidi; B) conidi isolati e catenulati.

Fig. 2.— *Aureobasidium pullulans* (De By.) Arn. (= *Trichosporium mantegazzae* Poll.). A e B) micelio con clamidospore e blastospore. C) Clamidospore seriate con blastospore.

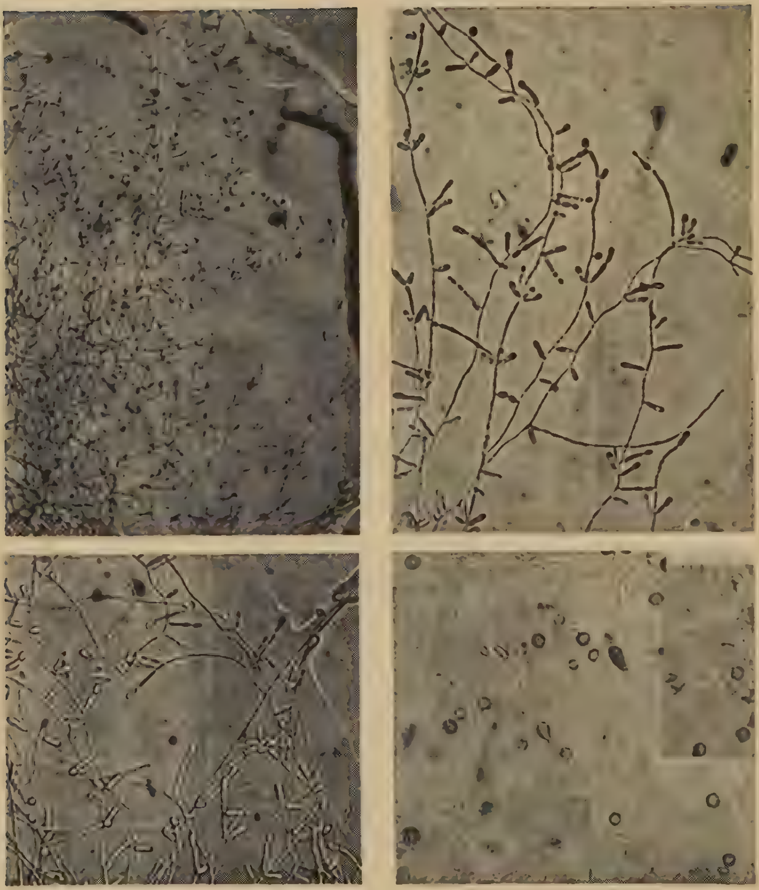


TAVOLA I.

Cladosporium chlamydeum n.sp.

In alto a sinistra: margine di colonia su Sabouraud mostrante l' aspetto generale del fungo.

In alto a destra e in basso a sinistra: dettagli di disposizione e forme di conidiofori e conidi.

In basso a destra: Clamidospore formate sulla superficie dell'agar.

spore sferiche sino ad ovoidi o piriformi ed anche clavate, sia intercalari che — raramente — apicali, a parete scabrida, bruna, abbastanza opaca, del diam. di 12—20 μ ma anche sino a 30—15 μ. La tunica è spessa e l'episporio è ornato di verruchette irregolari, quasi delle asperità, confuse.

Malgrado la scarsezza di conidi unisettati, l'aspetto del fungo è quello di una forma di *Cladosporium* a fruttificazione impoverita. Lo sviluppo dei conidi è quello descritto de De Vries (1952). La morfologia ricorda da vicino quella del *Cladosporium avellaneum* De Vries *f. viride* De Vries, un ceppo derivato dal tipo, mentre l'incremento in lunghezza dei conidiofori è considerato, per questo genere, da Hughes (1953). Nel nostro ceppo, però, si hanno inoltre le discretamente caratteristiche clamidospore miceliche, non segnalate dai due succitati autori.

Tra le specie a morfologia analoga, coltivate in substrati di laboratorio e sufficentemente ben descritte, il nostro fungo offre delle analogie con il *Trichosporium varium* Demelius (1923), isolato in Austria da un liquido („Aflin"), che però forma (su patata) colonie dorate, poi ferruginee ed infine nerastre. Ma i conidiofori, densamente (e non latamente) ramosi, sono spesso aggregati e spesso ramosi (mai nel nostro ceppo) ed i conidi possono giungere a formare anche dei glomeruli. Infine le clamidospore sono raffigurate come lisce.

E' possibile che il *T. varium* sia, in realtà, un *Cladosporium* a conidi lisci, ma in ogni modo differisce dal nostro ceppo.

La confusione esistente in merito alle specie del genere *Cladosporium* è inestricabile; anzi già il significato originale e la portata odierna del genere sono incerti. Così stando le cose, non possiamo se non proporre „ad interim" una nuova specie, in attesa di uno studio approfondito del genere che qualcuno dovrà pure affrontare: *Cladosporium chlamydeum* nob., n.sp. ad interim.

Coloniis ex agaro virido-griseis, dein nigridulo-viridatis, velutinis, versus fusco-olivaceus; mycelio parce ramoso, remote septato, ex hyphis subrectis, 2—3 usque 5—6 μ latis composito, hyalino, postremo brunneo; conidiophora lateraliter inserta, sparsa, pro more alterna, variabilia, typice cylindrica, sursum rotundata, basaliter attenuata, continua, hyalina, dein brunnea, 3,5—4,5 × 20—35 μ; conidia acrogena, singula, rarius 2-3-catenulata, ellipsoidea vel ovoidea aut piriculata, consuete continua (rarissime 1-septata), hyalina, dein brunnea, levia, pro more 3—8 × 3—5 μ; chlamydosporiis in agaro efformatis, rotundatis vel ovatis aut clavatis, pro more intercalaribus, 12—20 μ diam. vel usque 30 × 15 μ, fuscis, opacis, episporio brunneo, irregulariter asperato vel subverrucoso.

HAB.: ex epidermide canis dermatite eczematosa purulenta affecto, Italia.

Summary

Trichosporium mantegazzae Poll. from a human case, through the study of the original strain, has been recognized as a typical *Aureo-*

basidium pullulans (De By.) Arn. (formerly *Dematium* or *Pullularia*).

From the skin of a dog affected by a purulent, eczematous dermatitis, a species of *Cladosporium* has been isolated. It is described as a new species (*C. chlamydeum*) on account of the poorly developed, unbranched, as a rule monoconidial conidiophores, and for the chlamydospores produced in culture.

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SUCCESSFUL INFECTION OF PIGEONS AND CHICKENS WITH HISTOPLASMA CAPSULATUM

by

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(27.V.1957)

with 1 figure

Histoplasma capsulatum has been more frequently isolated from soil in the vicinity of chicken coops than from any other point source (1,7). Focal outbreaks (epidemics) of histoplasmosis were observed repeatedly in connection with cleaning of chicken coops (5) and handling of pigeon excreta (4). This has lead to the implication that chickens and pigeons might be connected in some way with the finding of *Histoplasma capsulatum* in the soil (2,12).

Search for *Histoplasma* in fowl has been so far fruitless, even if occasional positive skin tests with histoplasmin were obtained (8). Attempts to experimentally infect chickens with *Histoplasma capsulatum* have been unsuccessful except for one chicken injected by MENGES with packed yeast cells (9). This chicken died the day after inoculation and still had yeast cells in most organs on culture and microscopic examination. THOMISON failed to infect chickens by intraperitoneal, intravenous and intracerebral injection of yeast phase cells of this fungus (10).

AJELLO has stated that "*Histoplasma capsulatum* will not grow in vitro at 42°C., the normal body temperature of fowl" (11). In order to clarify the importance of birds and of temperature in the propagation of *H. capsulatum* the following studies were made:

1. The effect of a temperature of 42°C on *H. capsulatum* was studied in vitro.
2. Fecal material from chickens and pigeons was cultured.
3. Chickens and pigeons were infected intravenously with *Histoplasma capsulatum* and their organs cultured at autopsy and examined microscopically.

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Materials and Methods

1. Yeast phase cultures of three strains of *Histoplasma capsulatum* were grown on Kurung's egg medium, transferred to Sabouraud's broth and placed in the incubator at 42° C. (The temperature actually varied from 41.5° C to 42.3° C). At daily intervals a loop full of the well agitated broth was placed on Sabouraud's dextrose agar and kept for six weeks at room temperature (20—24° C.).

2. Guts from 115 chickens recently killed in a local commercial slaughterhouse were obtained and the fecal material removed. The feces from five animals were pooled and mixed with saline containing antibiotics in the concentration of penicillin 1000 units, and streptomycin 2000 units per ml. The 23 pooled samples were injected into 53 mice and 59 hamsters. These animals were sacrificed after 4—6 weeks and cultures made of their livers and spleens. Tissues were saved for microscopic examination. Droppings from the experimentally infected chickens and pigeons were treated in a similar manner and injected into hamsters. These animals were sacrificed after four weeks and cultures of the livers and spleens at autopsy were made as described above.

3. Three 6 weeks old tame pigeons, 41 wild pigeons and 10 two day old white rock roosters were injected intravenously with 0.1 ml of a suspension of the parasitic phase of *Histoplasma capsulatum* containing about 500.000 cells per ml. These animals were killed at intervals up to 45 days and the spleens, lungs and livers were cultured. Tissues were saved for microscopic examination.

Results

1. *Histoplasma capsulatum* remained viable and grew on subculture after 3 days exposure to 42° C.

2. Cultures from the guts of chickens were uniformly negative for *Histoplasma capsulatum*. All samples of chicken and pigeon feces were negative for *H. capsulatum*, but 13 of 20 fecal specimens collected from the pigeons of experiment 3 were positive for *Cryptococcus*. Of these, 8 were in pure culture and were identified as *Cryptococcus neoformans*. The other five were not checked because of gross contamination of the colonies. The frequent presence of *Cryptococcus neoformans* in droppings from pigeons in nature has been recently reported (3,6).

3. Positive cultures for *Histoplasma capsulatum* were obtained from the organs of 5 of the 10 inoculated chickens and from 20 of the 44 pigeons (Fig. 1). There was an obvious relationship between time of survival and positive cultures; all 5 positive chickens were killed within a week; the remaining 5 chickens which were killed after seven days were negative. Nineteen of the positive cultures in pigeons were obtained from animals killed within the first 24 days after inoculation; only one was positive after this time.

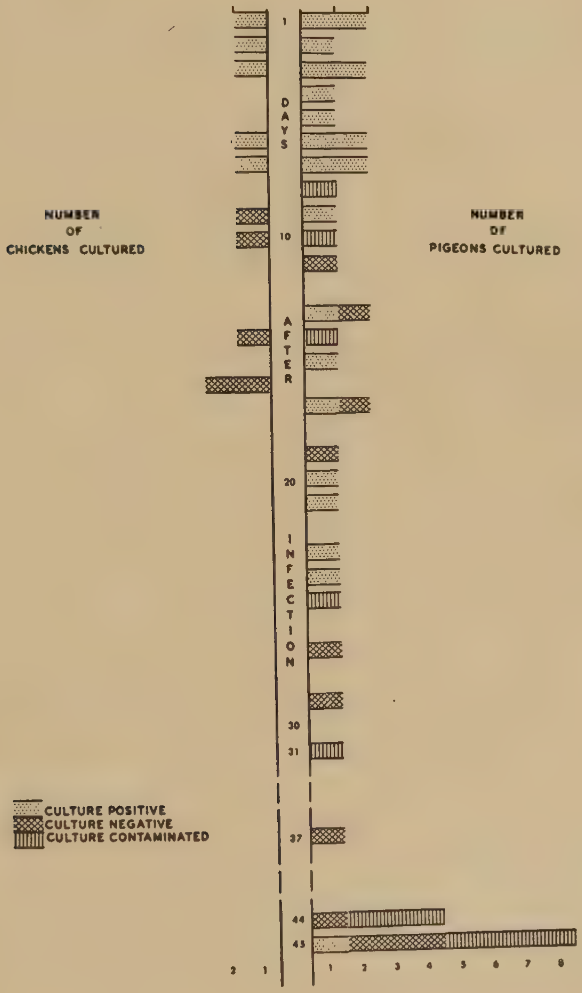

FIG. 1. *Results of culture from liver and spleen of birds after intravenous injection of Histoplasma capsulatum.*

Discussion

The results of the in vitro exposure of *Histoplasma capsulatum* would indicate that the organism can survive for a limited time at temperatures of 42° C, but his was true only for 1 out of 3 strains and only for a limited time.

However, infection of chickens and pigeons was accomplished with great ease and almost 50 % recovery of the organism from the internal organs of the animals was obtained, one as late as 45 days after inoculation. Little and inconclusive pathology was found in the organs of the birds and one tends to conclude that the animals at least do not become seriously ill from injection with *Histoplasma capsulatum* but are able to harbor it for a long time. It is of interest that only intravenous infection was successful; (ten chickens were infected intraperitoneally at the same time the intravenous inoculation was carried out and none was positive when killed at identical intervals as the intravenously infected chickens).

According to our temperature readings, the rectal temperature of 1—2 weeks old chicks varied from 40.2 to 41.6° C; pigeons had readings from 42.3 to 42.7° C.

The significance of our findings is that the birds tested *proved to be capable of carrying Histoplasma capsulatum* for a relatively long time and, therefore, are potential sources for the natural contamination of the soil. Whether or not histoplasmosis is a spontaneous infection of pigeons and chickens has not been settled by the present experiments.

Exactly how this applies to the previously observed association of chicken coops, pigeon excreta and epidemics of histoplasmosis is at present not clear.

The negativity of feces from birds for *Histoplasma capsulatum* would seem to indicate that simple direct fecal contamination of the soil is not the mechanism to explain the greater frequency of isolation of *Histoplasma* from soil around chicken houses.

Summary

The first repeatedly successful infection with *Histoplasma capsulatum* of pigeons and chickens is reported.

From 44 intravenously infected pigeons *Histoplasma capsulatum* could be recovered by culture in 20 cases as long as 45 days after injection; from 10 chickens 5 positive cultures were obtained.

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REVUE CRITIQUE DES INVESTIGATIONS ET DE LA LITTÉRATURE MYCOLOGIQUES POUR LES ANNÉES 1946—1956 EN GUYANE FRANÇAISE [1]

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(30.III.57)

Actinomycose

L'Actinomycose a été signalée par GUILBRIDE aux Caraïbes (1), où elle est plus fréquente chez les animaux que chez l'homme. Dans la zone intertropicale américaine, elle est retrouvée au Brésil et à Surinam, Trinidad et la Barbade, où l'on signale surtout des cas chez les animaux.

En mars 1953, un cas humain d'actinomycose a été diagnostiqué en Guyane Française (2). Il s'agissait d'un Indien Oyampi de l'Oyapock, âgé de 39 ans, qui avait présenté, en 1951, un abcès de l'hypocondre droit guéri après incision. Du pus, un *Bacille pyocyanique* et un *Staphylocoque doré,* furent alors isolés.

C'est en Janvier 1953 que le malade est de nouveau hospitalisé avec une température à 39° et une atteinte sérieuse de l'état général: on constate un abcès de même localisation. Après incision, du pus s'écoule et l'exploration montre une communication avec une lésion plus profonde.

Le pus, jaune verdâtre, révèle de nombreux petits bacilles gram négatif ne se développant seulement qu'en anaérobiose. Le bacille isolé est une variété d'*Actinomyces bovis*, pathogène pour le cobaye, chez qui il provoque une actinomycose expérimentale typique.

L'Hydrazide de l'acide isonicotinique per os à fortes doses (dans l'actinomycose vraie), et la Diaminodiphénylsulfone (dans le nocardiose) semblent les médicaments les plus actifs sur ces affections.

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[1] EDITOR'S note. — This is the first of a series of *Critical Reviews*, contributed by outstanding representatives of mycological research for *Mycopathologia et Mycologia Applicata* at the request of the Editor. These *Reviews* will cover practically every country on the globe where serious mycological research is going on. In this way we hope to present the results of mycological research in concentrated form, with global aims, including countries which are lesser known in the international community of mycologists, but made, nonetheless, substantial contributions to our knowledge. These *Reviews* should show the present state of mycological research in a given geographical area, its future trend and should be the source of mutual incentive for even higher goals to be achieved.

Histoplasmose

Depuis sa découverte par S. T. Darling (3) (4) en 1906, jusqu'en 1940, l'histoplasmose était considérée comme une maladie très rare et très grave. Localisée d'abord à la zone de Panama, cette affection est ensuite, reconnue dans de nombreuses parties des Amériques (5).

Da Rocha Lima (6) affirme, en 1912, la nature mycosique du parasite, et, W. A. de Montbreun réussit, en 1934, la culture d'*Histoplasma capsulatum* (7). Puis, grâce au test cutané à l'histoplasmine, des recherches aux Etats-Unis montrent que de nombreuses personnes réagissent positivement à l',,histoplasmine''. Le test cutané a mis en évidence la grande fréquence des formes bénignes et inapparentes de l'affection qui sont souvent en relation, semble-t-il, avec des calcifications pulmonaires, autrefois considérées comme tuberculeuses.

Au point de vue statistique et épidémiologique, l'intradermoréaction à l'histoplasmine a une réelle valeur, les réactions croisées avec d'autres antigènes mycosiques existant bien mais étant en somme loin d'être de règle.

L'histoplasmose est une maladie très répandue sur le Continent Américain mais n'existant pratiquement pas en Europe; seules de très rares exceptions ont été signalées. En Asie, en Afrique, et en Australie, la maladie existe mais avec une fréquence bien moindre qui n'a rien à voir avec celle rencontrée en Amérique, où, dans certains états des U.S.A., les 4/5 de la population réagissent positivement à l'antigène spécifique.

L'homme et les animaux semblent s'infecter à partir du sol, particulièrement par les voies respiratoires et digestives supérieures. Emmons & Negroni (8) ont obtenu l'isolement de *H. capsulatum* du sol.

L'histoplasmose est une réticulo-endothéliose chronique commune à l'homme et aux animaux.

Ceux-ci, le chien en particulier, semblent pouvoir être des réservoirs de virus.

H. capsulatum est un organisme arrondi, de 3 μ environ, colorable par le Giemsa; abondant dans les cellules fixes du foie et de la rate il est parfois phagocyté dans de grands mononucléaires.

A 37°, sur milieu au sang, on obtient des colonies formées d'éléments levuriformes (premier type cultural). A la température du laboratoire, sur milieu de Sabouraud, on a des colonies duveteuses, formées d'hyphes ramifiées portant trois types de spores (second type cultural). Certaines spores, arrondies, s'implantent directement sur les filaments; les autres, plus grandes, sont placées le long des hyphes; les dernières presque identiques aux précédentes ont une paroi superficielle recouverte d'excroissances verruqueuses.

Le diagnostic de l'histoplasmose est difficile, la symptomatologie étant très variée: fièvre, adénopathies, splénomégalie, anémie, troubles digestifs, ulcérations cutanéo-muqueuses, en sont les principaux symptômes. Ce diagnostic est confirmé par la mise en évidence des parasites dans la sérosité de ponctions ganglionnaire, sternale, splénique, dans le sang, dans les pus etc.

En Guyane Française, une étude systématique de la réaction à l'histoplasmine (9) a été effectuée en utilisant un antigène dilué au 1/1000. Parallèlement, une cuti-réaction à la tuberculine fut pratiquée chez chaque individu. L'influence raciale ne parait pas exister. En effet, tous les non-autochtones ayant des résultats positifs ont déjà vécu de nombreuses années en Guyane. Le nombre de réactions positives est identique (32 %) avec l'histoplasmino-réaction qu'avec la tuberculino-réaction. Mais l'examen approfondi des chiffres montre qu'il y a pas plus de coïncidences que ne pourrait le fournir le seul hasard.

Il n'y a pas plus de relation entre l'histoplasmino-réaction et la lépromino-réaction qu'entre histoplasmino-réaction et tuberculino-réaction.

Quand aux causes d'erreurs ou confusions possibles, relatives aux autres mycoses endémiques du continent américain (Coccidioidomycose, Blastomycose) elles ne semblent pas devoir être prises en considération pour des raisons de climat et de géographie.

Il n'existe aucune influence du sexe. Au point de vue de l'âge, de 10 à 20 ans, le pourcentage de positivité est de 36, identique à celui trouvé de 41 à 80 ans, ce qui tend à prouver que l'affection se contracte dans le jeune âge. Tous les sujets testés proviennent de la zone urbaine.

Le premier diagnostic d'histoplasmose humaine généralisée grave, en Guyane Française, a été porté en juin 1952, chez un jeune Créole, dont l'affection avait été diagnostiquée Maladie de Banti.

Parmi divers animaux sauvages, des recherches ont permis de rencontrer, chez un certain nombre d'entre eux, des histoplasmes. Il est difficile d'affirmer qu'il s'agit d'*H. capsulatum*, des cultures n'ayant pas été obtenues.

Il existe, en effet, de grandes différences morphologiques, notamment de dimensions, in vivo, entre les divers parasites décrits sous le nom d'*H. capsulatum*, mais certains auteurs reconnaissent aussi plusieurs formes morphologiques en provenance d'une seule souche de *H. capsulatum.*

Après avoir constaté qu'un tiers environ de la population guyanaise s'est trouvée en contact avec *H. capsulatum*, une enquête épidémiologique et, notamment, l'étude du réservoir de virus, a été effectuée (10).

ZEIDBERG et collaborateurs (11), cherchant dans le sol la solution du problème du réservoir de virus, ont entrepris une expérimentation importante, aux U.S.A.

En Guyane Française 160 échantillons de terres (prélevés en différentes régions du Département) de poulaillers en même temps que des antibiotiques (Streptomycine et Pénicilline) ont été inoculés à des souris et à des rats; cette terre a été prélevée, autant que possible, dans des recoins humides et au voisinage de trous de rats.

10 à 11 semaines plus tard, la recherche de *H. capsulatum* a été faite sur des frottis de foie et de rate, colorés au Giemsa, et, aussi, par ensemencements sur gélose glucosée de Sabouraud de fragments de ces mêmes organes. Sept souches de *H. capsulatum* ont été obtenues de cette façon; elles sont de provenances géographiques diverses.

H. capsulatum est donç bien présent dans le sol de toute la région côtière guyanaise.

Sur le frottis, il a souvent été possible de reconnaître deux groupes d'éléments, assez différents; en général il s'agit de corps ovalaires entourés d'une capsule et disposés en amas; parfois, on rencontre des éléments plus grands entourés d'une capsule à double contour.

D'après ZEIDBERG, certaines terres constituent un milieu plus favorable que d'autres au développement de *H. capsulatum*, ce sont les sols superficiels, acides, peu perméables, ne contenant que peu de matières organiques.

Si, en Guyane Française, la plupart des prélèvements ont été effectués sur sol sablonneux, il faut remarquer que tout le Nord-Est du Continent Sud-Américain est à classer dans le type ,,red-yellow podzolic'', sol de régions où ZEIDBERG a constaté les taux les plus élevés de tests positifs à l'histoplasmine. Quant à l'humidité il faut signaler que le degré hygrométrique ne descend jamais au dessous de 80 % en Guyane.

Par ailleurs le voisinage des trous de rat nous a paru favorable, au développement de *H. capsulatum.*

Le rat blanc parait nettement moins sensible que la souris blanche, mais il peut cependant être utilisé, pour l'isolement de *H. capsulatum.*

L'utilité d'un animal de laboratoire plus sensible à l'histoplasmine (tel le hamster doré) n'en est pas moins grande.

Les intradermo-réactions à l'histoplasmine nous ont donc montré 32 % de résultats positifs. La forme sous laquelle se manifeste la première atteinte par *H. capsulatum* doit passer bien souvent inaperçue (12).

L'hypothèse de l'infection naturelle d'origine respiratoire peut être retenue.

Divers auteurs ont montré que, bien souvent, un syndrome grippal pouvait être une primo-infection à *H. capsulatum.* La sensibilité à l'histoplasmine apparait vite après le début du syndrome (de quelques jours à 2 ou 3 semaines). C'est ce qu'ont constaté IBACH, LARSH & FURCOLOW (13) d'une part et SALVIN & FURCOLOW (14) de l'autre.

En décembre 1954 et janvier 1955, il y eut à Cayenne une épidémie de grippe. Or à la lecture des intradermo-réactions chez les enfants de l'Ecole Marchoux, on constata des réponses positives très fortes.

On relèva alors que ces enfants avaient présenté, quelques semaines auparavant, un épisode infectieux grippal avec fièvre, céphalée, toux, rhino-pharyngite à évolution souvent trainante. Une enquête plus élargie fut alors effectuée: toutes les personnes ayant présenté cette affection se montrèrent très fortement positives à l'histoplasmine; chez les autres individus, le pourçentage habituellement relevé chez les Guyanais fut retrouvé.

Ainsi l'épisode pseudo-grippal nous a paru un aspect important de la primo-infection par *H. capsulatum.*

Moniliase pulmonaire

En 1943, un cas de blastomycose pulmonaire a été diagnostiqué en Guyane (15).

M. S. est alors hospitalisé pour un point de côté très douloureux de l'hémithorax droit, de la fièvre et un amaigrissement marqué, sans symptômes pulmonaires. Au bout de 3 mois, avec l'apparition d'une toux accompagnée d'une expectoration séro-purulente et sanguinolente, le diagnostic est orienté vers une tuberculose évolutive.

L'examen bactériologique des crachats ne montre ni spirochètes, ni bacilles de Koch, mais de nombreuses levures. L'ensemencement sur milieux spéciaux a permis l'isolement d'un champignon levuriforme; une thérapeutique iodurée est alors instituée (10 injections intraveineuses de lugol). La guérison fut rapidement obtenue, et s'est maintenue par la suite.

Le champignon isolé des crachats a été étudié: la culture, blanc-jaunâtre sur milieux solides, montrait au microscope des formes levures arrondies ou ovalaires. Dans les cultures en cellule, on observait un bourgeonnement et une germination précoce des blastospores; le pseudomycelium était composé d'articles courts, dont le dernier prolongé par une chainette de blastospores.

De nombreux champignons ont été trouvés dans des crachats, mais les agents des blastomycoses pulmonaires sont soit des Ascomycètes, soit des Hyphomycètes. En l'absence d'asques, notre champignon fut rangé parmi les Hyphomycètes. Se reproduisant par blastospores et non par arthrospores il dut être classé dans le groupe des Blastosporés et d'après l'étude de LANGERON & TALICE (16), dans le genre Candida dont l'espèce type est *Candida tropicalis* (CASTELLANI & CHALMERS) (17).

Les formes graves de bronchomoniliase décrites par ces auteurs rappellent l'observation du malade X., avec son évolution rappelant la tuberculose, et l'efficacité du traitement ioduré.

Rappellons que GOMEZ, en 1912, au Brésil, a isolé un *Monilia butantanensis*, pathogène pour les animaux, et provoquant, chez l'homme, une affection à allure tuberculeuse. Quoique l'inoculation des crachats de M. X. n'ait pas été pathogène pour le cobaye, l'efficacité du traitement ioduré nous a permis d'attribuer un rôle pathogène spontané à ce champignon.

Une autre observation de Blastomycose pulmonaire dont l'évolution fut fatale a été diagnostiquée (18) en 1951. De très nombreux champignons ont été trouvés dans les crachats du malade.

LANGERON & TALICE (16) ont proposé, en 1932, en mycologie, une classification botanique basée sur l'aspect des cultures et l'appareil sporifère. Ainsi les champignons levuriformes sont rangés dans la sous famille des *Torulopsidées* (levures non filamenteuses) et celle des *Mycotorulées* qui est divisée en 2 groupes: *Geotrichoïdes* et *Mycotorula*, où doivent prendre place les ,,*Monilia*'' et les ,,*Mycoderma*''. Les champignons du genre *Candida* ont les caractères suivants: colonies crémeuses, longues chainettes terminales, verticilles simples.

CASTELLANI (19) et d'autres auteurs ont classé les *Monilia* (*Candida*) d'après les caractères biochimiques en 2 sous-genres: *Candida*, à cultures lisses et *Geotrichoides* à cultures granuleuses comportant de nombreuses espèces.

Chez notre second malade la *Monilia* du sous-genre *Candida* appartenait, d'après ses caractères botaniques, culturaux et biochimiques, à l'espèce *Candida tropicalis.*

N. R. C., 49 ans, est hospitalisé d'urgence pour une affection fébrile du type congestion pulmonaire. Il tousse, expectore, est amaigri: le diagnostic de tuberculose pulmonaire est alors évoqué. Les recherches de *Bacilles de Koch* ont été négatives, à 3 reprises et, 23 jours après son entrée, l'état général du malade s'aggrave; on note alors des crachats hémoptoïques; 3 jours plus tard il entre en agonie, et meurt.

Chaque fois, de nombreux champignons sont obtenus des expectorations, à l'examen direct et en culture.

Rappelons que du point de vue clinique, les moniliases bronchopulmonaires peuvent être, avec Castellani, divisées en 3 groupes.

Les formes bénignes qui ne présentent que des symptômes de bronchite apyrétique avec expectoration, l'état général restant bon;

Les formes graves qui simulent la tuberculose évolutive, avec des lésions pulmonaires manifestes, et dont la terminaison est fatale;

Les formes intermédiaires qui présentent les symptômes d'une bronchite chronique et qui peuvent être parfois l'aboutissement des formes bénignes.

Granulome cutané a Candida Albicans

Après ces cas de bronchomoniliases un granulome cutané à *Candida albicans*, affection bien plus rare encore, a été diagnostiqué en Guyane Française (20).

M. M., âgé de 46 ans, a depuis plus de 2 ans (début 1952), sur le dos de la main, une ulcération qui a été considérée comme une leishmaniose forestière; un traitement par la pénicilline et l'anthiomaline n'a donné aucun résultat favorable. Fin 1952, une petite lésion nouvelle apparait au bord cubital de l'avant bras droit: elle consiste en une induration qui s'ulcère; six autres petites lésions analogues apparaissent également sur le bord cubital de l'avant bras droit, s'ulcérant toujours superficiellement.

Lorsque nous examinons le malade on note des cicatrices sur l' avant bras droit ainsi que des traces de lésions anciennes sur la jambe gauche, et la paroi abdominale. Seule, la lésion initiale, placard granulomateux arrondi de 7 cm de. diamètre, est en activité. D'après M. M., le seul traitement, qui lui donne des résultats, consiste en badigeonnages iodés de la lésion.

Le diagnostic de leishmaniose est éliminé; le malade, en effet, habite St-Laurent, et ne va jamais ,,dans les bois''; de plus, le traitement d'épreuve, à l'anthiomaline a été négatif; enfin, la recherche de leishmanies a aussi été négative.

L'examen microscopique de la sérosité profonde de la lésion a, par contre, montré la présence d'une levure, obtenue en culture pure, sur milieu de Sabouraud. Cette levure a été identifiée comme *Candida albicans* (Robin 1853).

Le malade a été traité par un dérivé iodé (Thionaiodine) et la cicatrisation a été obtenue.

Blastomycose Sud Américaine

En 1955 nous avons rapporté un cas de Maladie de Lutz (21).

Cette Maladie de Lutz, ou Blastomycose sud-américaine, est une mycose caractérisée par des lésions granulomateuses atteignant muqueuses, peau, ganglions, tube digestif, poumons. L'agent pathogène est *Blastomyces brasiliensis* (22).

J. F., Créole, âgé de 45 ans, est dirigé sur l'Institut Pasteur de Cayenne, pour recherche de leishmanies en août 1954. Il aurait présenté, sept ans auparavant, un ,,pian-bois'', à l'épaule gauche, puis le nez est atteint, guérit, mais de nouvelles ulcérations sont constatées dans les fosses nasales.

A l'examen il est remarqué une cicatrice d'ulcération de la surface d'une paume de main sur l'épaule gauche, et, sur le nez, une cica trice rouge sombre, à gauche, avec ulcération de la narine du même côté, L'examen radiologique montre une ostéite condensante du massif facial.

Le laboratoire donne les renseignements suivants: Pas de leishmanies, pas de bacilles de Hansen, pas de tréponèmes, présence de nombreux éléments levuriformes bourgeonnants.

La nature pianique ou syphilitique de la lésion avait été également éliminée, tant par la clinique, que par la sérologie, et par un essai de thérapeutique par la pénicilline (échec).

Le diagnostic de leishmaniose a été discuté: le malade parle spontanément de ,,pian-bois''. Mais il n'a jamais été trouvé de leishmanies sur les frottis, nombreux, examinés à plusieurs reprises.

Les gros éléments levuriformes bourgeonnants sont donc un élément positif important en faveur du diagnostic de blastomycose sud-américaine, qui est finalement retenu.

Le diagnostic ainsi posé, nous avons recommandé un traitement à base d'iodure et de Disulone (Diamino-diphénylsulfone,), qui semble active sur certains champignons pathogènes. Le résultat obtenu a été très bon, puisque le 3 novembre, lorsqu'il quitte l'hopital, les lésions nasales entièrement cicatrisées, le malade dit se sentir guéri. Durant trois mois (du 3 août au 3 novembre) il a pris de la D.D.S à l'oxalate de fer à doses progressives (au début, 40 mgr. par jour pendant 15 jours, puis 80 mgr. par jour pendant 15 autres jours et ensuite 120 mgr. par jour), associée à la Naiodine (en injection intraveineuse de 10 cc. par jour), du 7 août au 4 octobre.

La Maladie de Lutz est une affection relativement fréquente en Amérique du Sud. O. VERSIANI & L. BOGLIOLO (23) en signalent mille cas environ au Brésil, où elle atteint surtout les hommes jeunes.

Le parasite des blastomycoses américaines, dans les tissus, est formé de cellules à double contour, se reproduisant par bourgeonnement unique ou multiple. Le bourgeonnement unique se rencontre chez *Blastomyces dermatitidis*, agent de la maladie de Gilchrist ou Blastomycose Nord-américaine, le bourgeonnement multiple se rencontre chez *Blastomyces brasiliensis*, agent de la Blastomycose sud-américaine.

Dans notre cas particulier il s'agissait de ce dernier agent pathogène.

Les cultures se présentent sous 2 aspects: à 37°, ce sont des colonies levuriformes; à la température du laboratoire ce sont des colonies filamenteuses.

Du point de vue clinique, il existe, considère-t-on, une forme localisée cutanéo-muqueuse (bucco-pharyngée) débutant par une petite papule qui s'ulcère, et une forme généralisée avec fièvre, amaigrissement et anémie.

L'histopathologie montre un granulome avec cellules géantes (renfermant les parasites), histiocytes, plasmocytes, lymphocytes, etc.

Les sulfamides sont très actifs.

Si, autrefois, la blastomycose sud-américaine était considérée comme une affection à pronostic grave, on s'est aperçu qu'il existait des formes bénignes, uniquement cutanées, pouvant durer des dizaines d'années avant de donner des généralisations viscérales: c'est la ,,Maladie de Lobo'' (24).

Chromoblastomycoses

Parmi les lésions cutanées provoquées par les champignons on rencontre fréquemment, en Guyane Française, des macules plus ou moins squameuses, plus ou moins surélevées, de teinte plus ou moins foncée, plus ou moins prurigineuses, appelées généralement ici ,,dartres bois'' (25).

De quelques prélèvements épidermiques, une fois, a été isolé un *Phialophora jeanselmi* (LANGERON 1928) ou une espèce très voisine. Plusieurs *Phialophora* sont admis comme agent de la chromoblastomycose, dont on a décrit divers types cliniques (CARRION & SILVA) (26):

1° — type nodulaire: nodules mous, de teinte foncée;
2° — type tumoral: masses papillomateuses;
3° — type verruqueux: masses nodulaires ou tumorales hyperkératosiques;
4° — type en plaque: macules squameuses surélevées;
5° — type cicatriciel: caractérisé par une guérison centrale, tandis que les lésions s'étendent à la périphérie.

Le type en plaque, défini comme ci-dessus, par ces auteurs, correspond bien, en vérité, à la lésion dont se plaignit notre malade, J. D., âgé de 37 ans, qui présentait une macule de 5 cm./2 cm., face postérieure de la cuisse droite.

Comme nous l'a précisé VANBREUSEGHEM, la systématique de ces levures noires est très confuse et leur signification ne l'est sans doute pas moins.

La chromoblastomycose est une maladie en somme rare (200 cas connus), rencontrée surtout à Cuba, à Porto-Rico et au Brésil, mais qui est, en fait, presque universellement répartie.

Dermatophyties

En 1942, un cas de kérion de Celse (25) a été diagnostiqué chez un enfant à Cayenne: un *Trichophyton* pyogène à culture blanche duveteuse, en a été isolé. Trois autres enfants de l'entourage ont présenté des lésions d'herpés circiné, d'où le même trichophyton *T. radians*, Sabouraud 1909, du groupe *Niveum*, a été isolé. La source d'infection était un jeune chat.

En juin 1954, de chez un homme de 38 ans atteint d'un sycosis de la barbe, fut isolé, par culture sur milieu de Sabouraud, un *Trichophyton mentagrophytes* du groupe *Gypseum*.

Pityriasis versicolor

Le *Pityriasis versicolor* (25), très fréquent en Guyane Française, est la mycose la plus banale.

Sur 504 personnes du sexe masculin que nous avons examinées 144 présentaient des lésions de *Pityriasis versicolor* (29 %); chez elles les localisations des lésions étaient par ordre d'importance décroissante: face 59 fois (40 %); tronc 42 fois (29 %); épaules et membres supérieurs 26 fois (18 %); cou 14 fois (10 %); fesses et membres inférieurs 2 fois (1 %); cuir chevelu 1 fois (0,5 %).

Sur 429 personnes du sexe féminin examinées, 93 étaient atteintes de Pityriasis (22 %); les localisations des lésions étaient, par ordre d'importance décroissante aussi: face 69 fois (74 %); tronc 18 fois (19 %); épaules et membres supérieurs 6 fois (6 %); cuir chevelu néant; cou néant; fesses et membres inférieurs néant.

Piedra noire

En octobre 1956, un cas de Piedra noire fut diagnostiqué, à l'Institut Pasteur, chez un jeune homme C. G., 25 ans, dont les cheveux sont assez longs et très abondants.

De nombreux cheveux étaient parasités et portaient de nombreux nodules piédriques, durs, noirs, irrégulièrement disposés, parfois groupés en chapelet (plus de dix par cheveu), parfois plus espacés.

On sait que la Piedra noire n'est connue qu'en Amérique du Sud: elle a été décrite en Colombie par DESENNE (27), puis au Vénézuéla, en Guyane Anglaise, au Brésil, en Argentine, en Uruguay et au Paraguay. Elle n'avait pas encore été signalée en Guyane Française.

C'est P. HORTA (28) qui, en 1911, fit le premier une description parfaite du parasite. BRUMPT & LANGERON (29) ont, ensuite, étudié ce champignon.

Après traitement à la potasse, on observe au microscope un stroma à structure radiée à la périphérie, avec, à l'intérieur, des asques ovalaires, contenant des corps fusiformes, allongés, prolongés à chaque extrémité par un flagelle: ce sont les ascospores, qui s'échappent de ces „kystes".

Ce champignon, du groupe des *Astérinées*, ne se développe que dans les climats humides. La Guyane Française, située dans la zone intertropicale, devait être intéressée par ce parasite.

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MYCOLOGIE MÉDICALE HUMAINE EN TUNISIE: REVUE DES PRINCIPALES ACQUISITIONS, ANALYSES DES DONNÉES CONCERNANT LES ANNÉES 1946—56.

par

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(13.V.1957)

Le Professeur T. Benedek a bien voulu nous demander d'établir une mise au point concernant les recherches mycologiques accomplies en Tunisie au cours de ces dix dernières années. La généreuse intention du Professeur Benedek d'augmenter et de faciliter les échanges entre techniciens s'occupant de mycologie dans les divers territoires, a rejoint notre désir de dresser un inventaire des acquisitions mycologiques médicales humaines en Tunisie, tant pour mieux voir le chemin parcouru que pour essayer de dégager certaines notions de caractère épidémiologique.

C'est pourquoi, avant de passer à l'étude des données d'acquisition récente, nous rappelerons brièvement quels ont été dès 1894, les agents mycosiques contractés en Tunisie, qui aient attiré l'attention des chercheurs et qui aient fait l'objet de publications.

C'est donc l'étude des mycétomes qui a principalement fait l'objet des premières recherches mycologiques en Tunisie. Si l'on se base sur le tableau précédent, il paraît possible de dire que l'extension des maduromycoses à *Streptomyces madurae* se limite dans ce pays, à la zone côtière et à la zone relativement montagneuse de la moitié Nord, et qu'elle n'atteint pas les zones franchement désertiques. Les cas de pied de Madura relevant d'une infection par Fungi imperfecti et par Ascomycètes sont par contre notés aussi bien dans des agglomérations de la côte Nord que dans les oasis sub sahariennes.

Au cours de cette dernière période (1946—1956), les recherches mycologiques ont non seulement le caractère sporadique du au hasard des observations cliniques, mais ces recherches donnent encore lieu à quelques sondages parmi la population tunisienne. Avant de dégager quelques vues d'ensemble, nous indiquerons par ordre chronologique ces diverses investigations.

En 1949, Moatti et col. (19) décrivent un nouveau cas d'actinomycose cervicale chez un malade qui présentait des grains jaunes dans les lésions. Mais les recherches mycologiques n'ont pas dépassé le simple examen extemporané des grains, et d'autre part le malade,

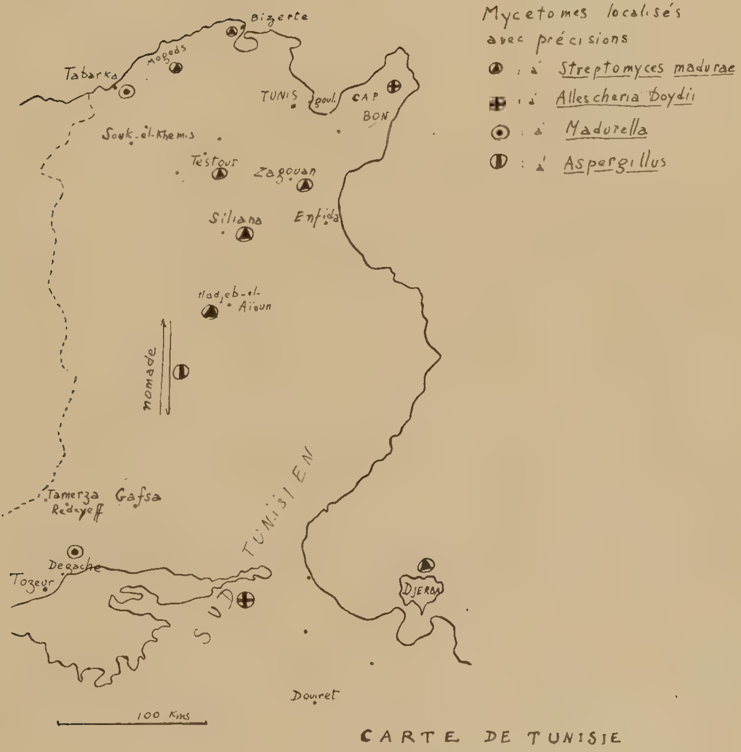
Mycetomes localisés
avec précisions
◭ : à Streptomyces madurae
⊕ : à Allescheria boydii
⊙ : à Madurella
◐ : à Aspergillus
Bizerte
Mogods
Tabarka
TUNIS
goul.
CAP
BON
Souk-el-Khemis
Testour
Zagouan
Siliana
Enfida
Hadjeb-el-
Aïoun
nomade
Tamerza
Redeyeff
Gafsa
Degache
Tozeur
SUD TUNISIEN
DJERBA
Douiret
100 Kms
CARTE DE TUNISIE

Fig. 1. Mycétomes localisés en Tunisie.

AGENTS MYCOSIQUES CONTRACTÉS EN TUNISIE, AYANT FAIT L'OBJET D'UNE IDENTIFICATION PRÉCISE

Espèces	Le malade	Le maladie	Contractée à	Le prélèvement	Ref
A l'origine de Mycétomes:					
Streptomyces madurae (Vincent 1894)	Homme, musulman, tunis. 40 ans.	Pied de Madura	Tunisie, sans précisions	Grains blanc-jaunâtre	1.
Streptomyces madurae (Vincent 1894)	Hom., tu., mus., 40 a.	Pied de Madura	Tunisie, sans précisions	Grains blancs	2
Streptomyces madurae	Hom., tu., mus., ?	Pied de Madura	Tunisie, sans précisions	Grains ?	3.
Streptomyces madurae (Vincent 1894)	Hom., tu., mus., 35 a.	Pied de Madura	Tunisie du Nord: Bizerte	Grains jaunâ.	4.
Streptomyces madurae (*Vincent* 1894)	Hom., tu., mus., 65 a.	Pied de Madura	Les Mogods	Grains blancs	2.
Streptomyces madurae (Vincent 1894)	Femme, tu., mus., ?	Pied de Madura	Testour	Grains blancs	3.
Streptomyces madurae (Vincent 1894)	Hom., tu., mus., 35 a.	Pied de Madura	Zagouan	Pus concrété	2.
Streptomyces madurae (Vincent 1894)	Hom., tu., mus., ?	Pied de Madura et extension lymphatique	Zagouan	Grains blancs	5.
Streptomyces madurae (Vincent 1894)	Hom., tu., mus., 25 a.	Pied de Madura	Siliana	Grains blancs	3.
Streptomyces madurae (Vincent 1894)	Hom., tu., mus., 35 a.	Pied de Madura	Tunisie centrale: Hadjeb-el-Aïoun	Grains jaunâ.	6.
Allescheria boydii Shear 1921	Hom., tu., mus., ?	Pied de Madura	Sud tunisien, sans précisions	Gr. bl.-jaunâ.	3.
Allescheria boydii Shear 1921	Hom., tu., mus., 30 a.	Pied de Madura	Cap Bon	Gr. blancs	7.
Aspergillus nidulans (Eidam 1883)	Fem., tu., mus., 40 a.	Pied de Madura	Nomade	Gr. blancs	4.
Madurella tabarkae Blanc et Brun 1919	Hom., tu., mus., 30 a.	Pied de Madura	Tunisie du Nord: Tabarka	Grains noirs	4.

AGENTS MYCOSIQUES CONTRACTÉS EN TUNISIE, AYANT FAIT L'OBJET D'UNE IDENTIFICATION PRÉCISE

Espèces	Le malade	La maladie	Contractée à	Le prélèvement	Ref
Madurella tozeuri Nicolle et Pinoy 1908	Hom., tu., mus., 45 a.	Pied de Madura	Sud tunisien: Degache	Grains noirs	4.
Espèces á l'origine de syndrômes divers					
Aspergillus tunetanus (Langeron 1924)	Hom., tu., mus., 60 a.	Tumeur ulcérée main, adénopathies	Sud tunisien: Tozeur	Ponction de ganglions fermés	8.
Aspergillus niger Van Tieghem 1877	Fem., franc., ?	Otomycose	Tunisie du Nord: Tunis	Squames conduit auditif	9.
Geotricum brocianum Pinoy 1930	Hom., tu., mus., ?	Lésions ulcéronodulaires main et avant bras	Tunisie sans précisions	Ponction de nodules fermés	10

AGENTS MYCOSIQUES CONTRACTÉS EN TUNISIE, AYANT FAIT L'OBJET D'UNE IDENTIFICATION APPROXIMATIVE

Actinomyces israeli?	Fem., tu., mus., 45 a.	Pied de Madura	Tunisie sans précisions	Grains jaunes	11.
	Hom., franc., 44 a.	Actinomycose cervico- faciale	Tunisie du Nord: Souk el Khemis	Grains jaunes	12.
	Hom., tu., mus., 33 a.	Actino. cervicofaci. et thoraci.	,,citadin''	Grains jaunes	13.
Madurella?	Fem., tu., mus., 35 a.	Lésions jambes	Tunisie du Sud: Tozeur	Particules noirâtres	4.
	Fem., tu., mus., 19 a.	Lésions pied, jambe, main	Gafsa	Corps noirs	4.
Madurella tabarkae?	Hom., tu., mus., 30 a.	Pied de Madura	Douiret	Grains blancs	14.
Candida groupe *krusei?*	Hom., franc., 3 mois	nodules ulcéres occipitaux, cervicaux, inguinaux	Tunisie du Nord: Tunis	Ponction nodules fermés	15.
Candida groupe *tropicalis?*	Hom., tu., mus., ?	ulcérations et abcés bras	Tunis-Goulette	Ponction abcés fermé	16.
Achorion schönleini?	tu., mus.	Favus généralisé aux parties glabres	courant en Tunisie		17.

A signaler un cas de sporotrichose suspectée mais n'ayant fait l'objet d'aucun examen bacteriologique. 18.

d'origine européenne, aurait pu contracter son affection hors de Tunisie. Ce cas pourrait donc être la 4ème observation tunisienne probable de mycétome du à *Actionomyces israeli* (Kruse 1896).

En 1951, Durand isole du L.C.R. d'un malade ayant présenté un syndrôme méningé, une souche de *Cryptoccocus neoformans* (San Felice 1894). Cette souche est l'objet d'une expérimentation poussée sur animaux dont l'étude histologique des lésions est faite par Gordeeff. Mais il ne semble pas, à notre connaissance, que ces auteurs aient communiqué directement les résultats de leurs travaux, qui ont fait l'objet d'une thèse (Rault) (31). C'est toujours au cours de cette même année que Ottavy (20), Jadfard (21) contribuent à délimiter le problème des teignes en Tunisie (6500 cas évalués en 1950 sur une population de 160.000 écoliers). Ottavy souligne la rareté des teignes faviques en milieu scolaire, Jadfard l'extrème rareté, dans le même milieu, des teignes à *Sabouraudites audouini* (Gruby 1843), sans toutefois apporter ni éléments statistiques, ni éléments d'ordre technique permettant d'affirmer cette détermination d'espèce.

En 1952, nous avons l'occasion (22) d'étudier l'agent d'une mycose généralisée mortelle, dont les cliniciens Broc et Haddad (23 et 24) ont donné l'observation clinique complète. Le syndrôme décrit paraît bien se rattacher à la ,,Systemic Blastomycosis'' des auteurs Nord-américains, cas particulier de la maladie de Gilchrist. Mais primitivement influencé par l'opinion de Langeron qui attribue aux travaux des auteurs japonais Ota et Kawatsure (25) une importance décisive quant à la conception que l'on doit se faire de l'étiologie de la maladie de Gilchrist, nous sommes amenés à étiqueter l'agent causal de ce cas tunisien *Scopulariopsis americana* Ota 1926. C'est cette dénomination qu'adoptent, sur nos indications, les cliniciens dont l'un d'eux complète sa thèse de médecine à partir de nos expérimentations. Mais par la suite, sur les conseils de Segretain, nous revenons sur notre première détermination et préférons l'étiquette actuellement à peu près universellement admise de *Blastomyces dermatitidis* Gilchrist et Stokes 1898. En effet il nous paraît difficile d'appeler phialide, élément pourtant susceptible de réduction, le fin tractus porteur de spores, et de classer ce germe parmi les Aspergillacées. Segretain, Ajello, Van Uden, chercheurs qui ont des subcultures de cette souche isolée à partir de ce cas tunisien, pourront peut-être apporter de nouveaux éléments quant à la définition de ce germe. Retenons que l'inoculation de la phase filamenteuse de cet agent mycosique, quels que soient la voie et l'animal utilisé, détermine l'apparition de lésions histologiques spécifiques contenant la phase parasitaire levuriforme.

Nous nous sommes demandés quelle pouvait être éventuellement l'extension de cette nouvelle affection pour la Tunisie, dans la région où la malade l'avait vraisemblablement contractée. Nous nous adressons alors à la méthode immunologique d'investigation qui consiste à suivre la réaction provoquée par l'inoculation intradermique d'un antigène. Nous utilisons deux blastomycétines, l'une préparée à partir de la souche isolée en Tunisie, selon la méthode préconisée par EMMONS (26), l'autre telle qu'elle est préparée par la Société PARKE DAVIS & COMPANY (Detroit, Michigan), que nous remercions vivement de son gracieux envoi. Chaque blastomycétine est appliquée, en 1955, sur 50 personnes de nationalité tunisienne, en provenance de la région minière (exploitation de phosphates aux environs de Gafsa), ainsi que de la région désertique de la Tunisie. Nous n'obtenons, de ces 100 épreuves, aucune réaction vraiment positive. Si l'on ne peut pas tirer de conclusion valable de ce sondage, il est possible de dire que les mycoses de type ,,Systemic Blastomycosis'' ne paraîssent pas poser de problèmes imminents à la Santé tunisienne. Une tentative d'application sur une plus grande échelle d'un tel système de recherches sur les lieux mêmes où la malade a contracté son affection, se heurte à l'hostilité des médecins des compagnies minières.

En 1956, LADJIMI et KHALFAT (27) publient l'observation clinique d'un cas de pied de Madura dont l'agent infectieux est étudié par MARIAT et nous-même et étiqueté *Streptomyces madurae*. Le malade, Tunisien de 46 ans, a contracté son affection dans l'île de Djerba, et il est porteur au niveau des lésions de grains blanc-jaunatre. Le germe présente, à l'isolement, un comportement microaérophile qui se transforme en aérobiose franche au 2 ème repiquage, ce dernier caractère durable jusqu'à présent. Ce germe survit 3 semaines, sans faire de grains, dans le péritoine d'une gerbille (*Gerbillus hirtipes* LATA.) inoculée. A noter que MACKINNON et col. (28), étudiant les caractéristiques de plusieurs souches de *Streptomyces madurae*, utilisent une des souches isolées par NICOLLE en Tunisie, souche qui est conservée à l'Institut O. Cruz.

Toujours en 1956, l'Ecole parasitologique de Lille, que dirige le Professeur COUTELEN (29) publie les résultats d'une étude considérable entreprise dès 1950, sur la connaissance épidémiologique et mycologique des teignes infantiles de Tunisie. L'enquête porte sur plus de 24.000 enfants, essentiellement des écoliers musulmans. Près de 2500 prélèvements sont positifs. Les auteurs concluent que les teignes ne sont représentées que par des favus et des trichophyties, les microspories et les teignes suppurées sont exceptionnelles. Sur 1.623 souches, *Trichophyton violaceum* BODIN 1902 est isolé 1.050 fois *Achorion schönleini* (REMAK, 1845) 546 fois. Les autres espèces sont représentées par *Trichophyton tonsurans* (MALMSTEN 1845) (17 souches généralement atypiques), *Langeronia soudanensis*. (JOYEUX 1912) (1 souche), *Ctenomyces mentagrophytes* (ROBIN 1853) (6 souches dont 2 atypiques) et *Sabouraudites canis* (BODIN 1897) (3 souches).

Dans l'ensemble, les villes côtières sont reconnues moins parasitées que les villes et villages de l'intérieur. Au cours d'une première campagne, il est observé trois fois plus de teignes trichophytiques que de teignes faviques, mais ce rapport de fréquence s'inverse à Kairouan, ce qui semble, dans une certaine mesure rejoindre l'assertion de certain dermatologue tunisois qui nous disait en 1949 que les trichophyties disparaîssaient au niveau de cette ville sainte. Mais au cours d'une deuxième campagne menée en grande partie dans le Sud tunisien, il est trouvé, à part quelques localités d'exception, toujours le même rapport que dans les villes côtières (rapport de répartition des divers types de teignes) où les enquêtes de la première mission ont eu lieu.

Cette même Ecole (30) conclue, à partir de l'étude de 437 souches de trichophytons glabres d'origine tunisienne, que *Trichophyton glabrum* n'a aucune individualité réelle, même en tant que variété, et que les cultures blanches des trichophytons glabres ne représentent que l'un des aspects morphologiques possibles des cultures de *Trichophyton violaceum.*

Pour compléter cette étude des dermatophytes rencontrés en Tunisie, signalons l'isolement en 1952, à partir d'une trichophytie blépharo-ciliaire chez un jeune israëlite du port de la Goulette (Tunis), d'une souche de *Trichophyton tonsurans* dont le développement des caractères culturaux correspondait très exactement à la description donnée de *Trichophyton sulfureum* COL. FOX 1908. Il s'agit peut-être là d'une mycose d'importation. A noter, en outre, et bien que cela dépasse le cadre de cette étude, la présence à Tunis d'épizooties à *Trichophyton quinckeanum* (ZOPF 1890) affectant la souris commensale *Mus musculus brevirostris*. Ces épizooties sont assez rarement observées mais on les retrouve périodiquement.

Est encore en cours d'étude un cas de mycose généralisée au système lymphatique dont nous sommes en droit d'attribuer l'origine à *Aspergillus fumigatus* FRÉSÉNIUS 1863. Le malade, Tunisien d'environ 12 ans, venant de la plaine centrale de l'Enfida, a été hospitalisé à Tunis en 1954, considéré au départ comme pulmonaire sans étiquette bien précise. Ce malade présente des manifestations lymphatiques successives, cervicales, thoraciques et abdominales, se traduisant par l'apparition de chaines de ganglions hypertrophiés. C'est à partir de l'étude histologique (GORDEEFF) des premiers ganglions cervicaux hypertrophiés que nous sommes guidés vers la diagnose de l'affection. En effet ces ganglions présentent des cellules géantes contenant de rares éléments mycosiques mycéliens d'interprétation délicate. Mais à partir de nouveaux ganglions présentant un stade plus avancé d'infection, mais toujours fermés, nous pouvons retirer par ponction une masse nécrotique contenant des amas mycéliens évidents et isoler une souche *d'Aspergillus fumigatus* (détermination confirmée par SEGRETAIN). Ces derniers ganglions ont perdu leur structure normale. Ce cas sera l'objet, collaborant avec L'Ecole de SEGRETAIN, d'une étude avec documentation micro-photographique pour montrer la réalité de la pathogénicité de l'ASPERGILLUS. Cette observation peut être considérée la 2ème en Tunisie, de localisation ganglionnaire d'un *Aspergillus.*

Cette dernière période (1946—56) d'étude mycologique en Tunisie est donc caractérisée non seulement par l'étude poussée des dermatophytes, mais aussi par l'étude des agents de ce que l'on appelle les grandes mycoses viscérales mortelles. Ce dernier groupe peut encore réserver d'intéressantes constatations, il n'a pas encore été signalé, du moins à notre connaissance, de cas d'histoplasmose, affection probable en Tunisie (nous observons, en 1952, sur des frottis provenant d'un prélèvement abdominal fait chez une Tunisienne, des images rappelant bien le genre *Histoplasma*, mais l'absence de culture nous interdit de tirer parti de cette observation). A signaler, en outre, la contribution tunisienne à l'étude de la pathogénicité de certaines Aspergillacées.

Toutes ces études ont eu un point de départ clinique, il n'a pas encore été fait en Tunisie de recherches sur l'origine dans la nature, d'agents mycosiques pathogènes pour l'homme.

Nous donnons dans un tableau l'énumération des agents mycosiques contractés par l'homme en Tunisie, ayant fait l'objet d'une identification précise et d'une publication:

ACTINOMYCETES	ASCOMYCETES	FUNGI IMPERFECTI
Streptomyces madurae	*Allescheria boydii* *Aspergillus nidulans* *Aspergillus tunetanus* *Aspergillus niger* *Aspergillus fumigatus* *Cryptococcus neoformans* *Achorion schönleini* *Trichophyton violaceum* *Trichophyton tonsurans* *Langeronia soudanensis* *Ctenomyces mentagrophytes* *Sabouraudites canis*	*Madurella tabarkae* *Madurella tozeuri* *Blastomyces dermatitidis* *Geotricum brocianum*

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DESARROLLO DE LOS ESTUDIOS MICOLÓGICOS EN LA ARGENTINA EN EL ÚLTIMO DECENIO

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(9. VII. 57)

Este artículo viene a completar nuestra ,,Reseña histórica de la micología médica Argentina'' publicada en Mycopathologia, **1**, 267, 1939. Además el Ing. Juan B. Marchionatto dió a conocer en 1947 la evolución de los conocimientos de la micología pura y aplicada a la fitopatología (An. Soc. Cient. Argentina, **143**, 14-20, 1947).

En el último decenio vieron la luz unos cuatrocientos trabajos sobre micología, representando más de un tercio de los mismos los de aplicación médica, siguiéndole, luego, los de fitopatología.

Los estudios de micología médica han permitido obtener los siguientes datos de las micosis superficiales y profundas.

Micosis superficiales.

Negroni y colaboradores registraron 1.505 casos de micosis superficiales en el período comprendido entre los años 1949-1953 en el Centro de Micología de Buenos Aires con la siguiente distribución etiológica: dermatofitias 1.107 casos; infecciones por blastosporados (la mayoría por *Candida*) 337; micosis diversas (eritrasma, pitiarisis versicolor, piedra) 61 casos.

Las dermatofitias comprendieron las localizaciones siguientes: 615 casos de tiñas del cuero cabelludo; 226 dermatofitias de la piel lampiña; 50 epidermofitias de los grandes pliegues; 74 eczemas micósicos de los pies; 24 de las manos y 98 casos de onixis tricofíticas.

La mayoría de las tiñas son microspóricas (72,92 %); 23,73 % tricofíticas y 1,78 % fávicas. El agente de la tiña microspórica es, en la Argentina, el *Microsporum canis*. Solamente en un caso obtuvimos el cultivo del *M. gypseum*. El. *M. canis* está, en la Argentina, tan adaptado al parasitismo que se comporta como el *M. audouini* en Europa y los EE.UU., es decir, produce lesiones no inflamatorias, habiendo perdido su caracter zoófilo.

Sobre un total de 92 cultivos de *Trichophyton* obtuvimos la siguiente distribución por especies: *T. violaceum* 30; *T. rubrum* 29; *T. mentagrophytes* 23; *T. schoenleini* 3; *T. flavum* 1 y 6 cultivos no

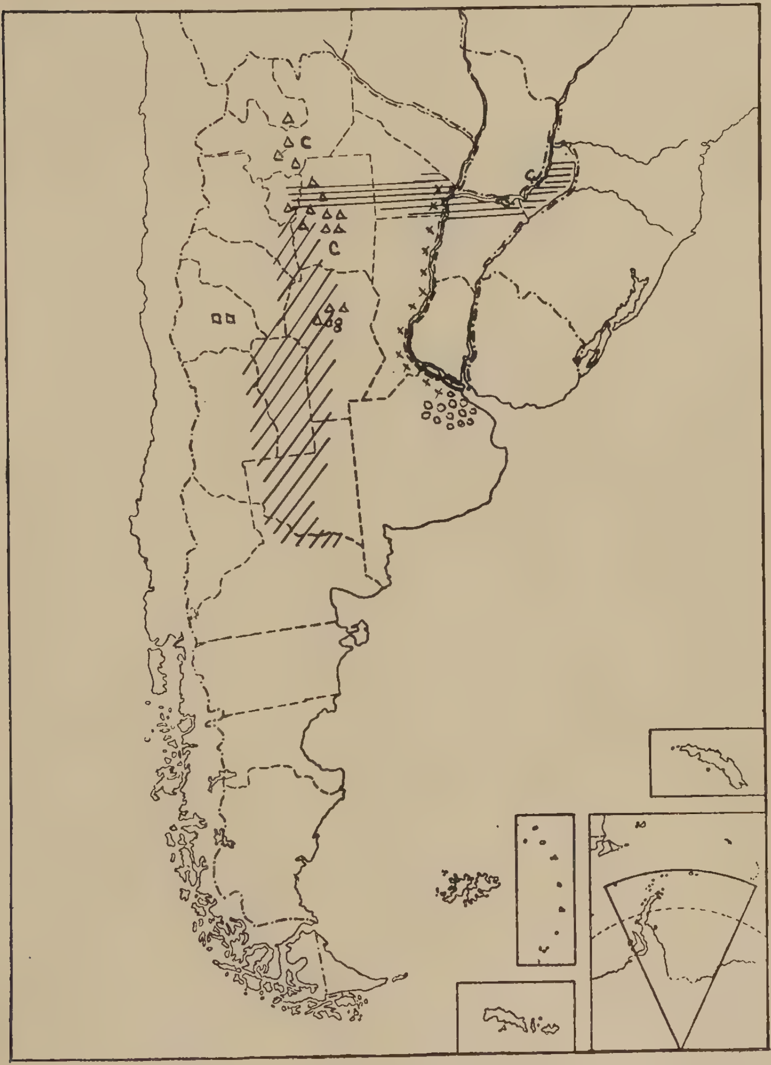

Fig. 1. — Distribución geográfica de las micosis profundas en la Argentina.

≡ Zona endémica de blastomicosis sudamericana

////// Zona endémica de coccidioidomicosis

oo oo Incidencia de la histoplasmosis

xx Incidencia de la Rhinosporidiosis

△ Incidencia de las mudurомicosis

c Casos de cromomicosis

□ Casos de trichosporosis pulmonar

identificados. Estos cultivos proceden de todas las dermatofitias y no solamente de casos de tiña del cuero cabelludo.

Queremos hacer notar el siguiente hecho interesante: en nuestra casuística del año 1942, casi 40 % de las especies de *Trichophyton* correspondían al grupo del *T. mentagrophytes* y solamente 3,33 % al *T. rubrum,* en tanto que, en la actualidad, encontramos 31,52 % de *T. rubrum* sobre 25 % del grupo *mentagrophytes.* Esta desviación de la frecuencia relativa de estos *Trichophyton* ha sido también observa en el Skin and Cancer Unit de Nueva York y posiblemente se deba a una mayor resistencia del *T. rubrum* a los nuevos agentes terapéuticos empleados corrientemente en el tratamiento y profilaxis de las dermatofitias.

Las onixis blastomicéticas representaron 82,79 % de las infecciones por blastomicetes procedentes de consultorios dermatológicos; 11,86 % intertrigos y 6,23 % localizaciones mucosas.

El agente más frecuente de las infecciones por blastosporados es *Candida albicans* (75 % de las especies), habiéndose identificado, también, *C. solani, C. parapsilosis* y *Torulopsis famata.*

Negroni y Briz de Negroni („Symposium" sobre Moniliasis, Buenos Aires, Octubre de 1956) comunicaron los resultados obtenidos con el siguiente medio de cultivo: peptona 1 %; inulina y dextrina 0,1 % de cada una; fosfato monopotásico 0,1 %; sulfato de magnesio 0,05 %; gelatina 12 % y agua destilada c.s.p. 100 cc. sembrando el material del cultivo aislada, por punción. El desarrollo de *C. albicans* tiene el aspecto de un pino invertido, al cabo de 4 a 5 dias de incubación a 22° y el examen microscópico permite apreciar la existencia del seudomicelio típico con los racimos globulosos de blastosporos y numerosos clamidosporos.

Los casos de piedra negra y blanca son poco numerosos habiéndose idenficado los siguientes agentes: *Piedraia hortai, Thichosporon beigeli* y *T. ribeiroi.*

En otras zonas de la Argentina, la flora de las micosis superficiaales tiene, aproximadamente, los mismos caracteres.

Vallejo y Rodriguez (1949 y 1956) registraron en el norte Argentino (Tucumán) una incidencia de 9, 78 % de micosis superficiales sobre un total de 4.927 pacientes dermatológicos de la clínica privada. Las microsporias representan 71, 20 % de las tiñas del cuero cabelludo (sobre 104 casos) y 28,80 % las tricofíticas siendo más frecuentes en el varón y entre los años 4 y 8 de edad. El *M. canis* es, igualmente, el agente habitual de la tiña microspórica. Solamente en un caso de tiña inflamatoria, tipo zoófilo, obtuvieron el cultivo del *M. gypseum.* Estos investigadores identificaron, también, las siguientes especies de *Trichophyton*: *T. tonsurans, T. mentagrophytes, T. plicatile, T. flavum, T. discoides, T. violaceum, T. acuminatum* y *T. rubrum.*

Tello, E. (1950) en un estudio efectuado en el centro del pais (Córdoba), comprobó que la tiña microspórica representa 97, 20 % de los casos, siendo ocasionada, habitualmente, por el *M. canis.*

Respecto a la tiña tricofítica comprobó una incidencia de 2,78 %.

La casuìstica de Vallejo y Rodriguez acusa, además, la siguiente frecuencia de otras micosis superficiales: epidermofitias de los pies 35, 3 %; epidermofitia inguinal 9,1 %; herpes circinado 9,3 %; eczemas paraqueratósicos blastomicéticos 3,5 %; onixis blastomicéticas 4,5 %; intertrigos blastomicéticos 11, 6 %; pitiriasis versicolor 18 %; eritrasma 1,4 % y piedra 0,2 %.

Micosis profundas.

En nuestras observaciones comprendidas entre los años 1949 y 1953 registramos 73 casos de micosis profundas con la siguiente distribución: actinomicosis 25 casos, blastomicosis sudamericana 30, blastomicosis europea 1 caso, histoplasmosis 6 y maduromicosis 4.

En los 3 años posteriores registramos 16 casos de blastomicosis sudamericana, 7 de actinomicosis, 2 casos de trichosporosis pulmonares, dos de maduromicosis y uno de histoplasmosis.

En 1952 Negroni publicó la siguiente clasificación de las micosis profundas, teniendo en cuenta sus caracteres clínicos y etiológicos: Se reconocen, ante todo, dos grandes grupos: I, debidas a hongos de acción patógena reconocida y II, debidas a hongos de acción patógena dudosa u ocasional. Grupol: 1) Micosis localizadas en las partes descubiertas del cuerpo; 2) Micosis tumorales con traycetos fistulosos por los cuales se elimina pus con granos: Micetomas. Los madurомicósicos propios del hombre y sin tendencia a propagarse por la via vascular y los micetomas actinomicósicos comunes al hombre y a ciertos animales que pueden propagarse por la vía sanguinea. 3) Micosis granulomatosas o supurativas de comienzo cutáneo, mucoso o pulmonar debidas a hongos que en la vida parasitaria y, algunos también en los cultivos, se presentan como células brotantes: las blastomicosis. La blastomicosis europea por *Cryptococcus neoformans* blastomicosis norteamericana por *Blastomyces dermatitidis* y la sudamericana por *Paracoccidioides brasiliensis*.
4) Micosis gomosas y ulcerosas polimorfas que tipicamente se propagan por la via linfática y a veces, también, por la sanguinea producidas habitualmente por el *Sporotrichum schenckii* y ocasionalmente por el *Trichosporon cutaneum* y otros hongos.
5) Micosis que ataca el sistema reticuloendotelial: Histoplasmosis.
6) Micosis de comienzo pulmonar generalmente benigna, asintomática o subclínica: Coccidioidomicosis o enfermedad de Posadas.

Otras micosis pulmonares: Por *Trichosporon, Aspergillus fumigatus*, etc.

Grupo II: Se colocan las micosis tumorales (maduromicosis) producidas por *Aspergillus, Penicillium*; las micosis úlcerogomosas por *Scopulariopsis, Hemispora, Acladium* etc.; las micosis broncopulmonares localizadas o diseminadas por *Candida albicans,Penicillium, Mucor*, etc.

Nos ocuparemos sucesivamente de las distintas micosis profundas siguiendo el órden expuesto.

Respecto a las micosis localizadas, de preferencia en las partes descubiertas del cuerpo, comprenden la Rhinosporidiosis y la cromomicosis.

La **Rhinosporidiosis** parece exister endémicamente a lo largo del Rio Paraná, desde la ribera del Plata hasta el Chaco. Se conocen unos 15 casos humanos y otros tantos en animales domésticos: mulares y equinos. Recientemente MEYER y DIAZ señalaron casos en el perro y el vacuno en Resistencia (Chaco).

La cromomicosis se presenta en forma esporádica en el norte argentino, producida por *Fonsecaea pedrosoi.*

La **enfermedad de Jorge Lobo** o blastomicosis queloidica e desconocida en la Argentina.

Los Micetomas: NEGRONI le dedicó, en 1954, el primer volumen de las micosis profundas. Fué editado en Buenos Aires por El Ateneo y consta de 387 paginas, 109 figuras, indice aflabético y bibliografía al final de cada capítulo. De los 17 capítulos en que está dividída la obra, el primero está dedicado a las consideraciones generales sobre las micosis profundas, capítulos II al XV tratan de las diferentes localizaciones de la actinomicosis y de sus agentes etiológicos. Finalmente los capítulos XVI y XVII están consagrados a las maduromicosis.

Nuestra casuistica de 120 casos de actinomicosis observados en la ciudad de Buenos Aires, permite apreciar una frecuencia relativa de sus diferentes localizaciones que coincide con la de otros paises templados o frios: localización cefálica 61, 66 %: torácica 20 %; abdominal 4,16 %; miembros inferiores 7,5 % y otras localizaciones 6,66 %. El agente habitual de las localizaciones cefálica, torácica y abdominal es el *Cohnistreptothrix* (Actinomyces) *israeli* y el más frecuentemente encontrado en las actinomicosis de los miembros, el *Actinomyces* (Nocardia) *asteroides.*

Respecto a las maduromicosis se conocen apenas una decena de casos producidos en el norte y centro del pais casi todos por *Madurella*, uno por *Monosporium apiospermun* (JORGE y colaborades, 1941) y otro por *Pseudallescheria sheari* (NEGRONI y colaboradores, 1944). Se han señalado en la Argentina las siguientes especies de *Madurella*: *mycetomi*, *ramiroi* y *tabarkae,* siendo la primera la más frecuentemente encontrada.

La **blastomicosis europea** se presenta en forma esporádica, la mayoria de las observaciones proceden de Buenos Aires y de Córdoba. La localización en el sistema nervioso central (forma meningea o tumoral) ha sido la más frecuentemente registrada, habiéndose observado casos con localización pulmonar, cutánea o ganglionar simulando la enfermedad de Hodgkin.

Blastomicosis sudamericana: es endémica en la Argentina en la región noreste, húmeda y boscosa continuándose con la zona de endemia brasileña y paraguaya, de clima subtropical. La zona mas

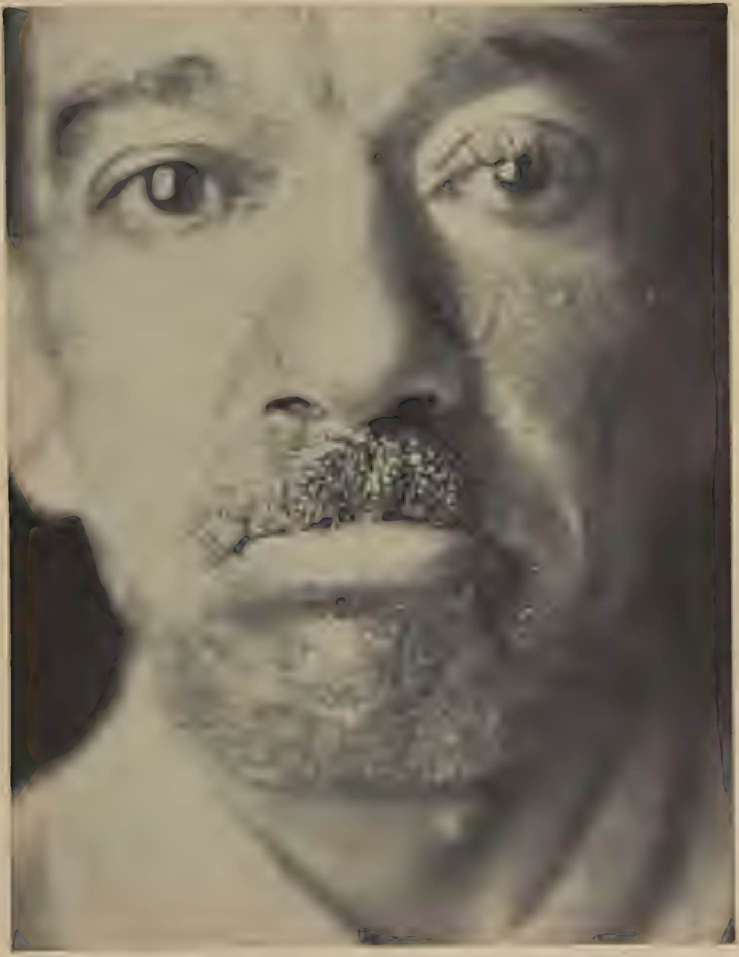

Fig. 2. – Lesiones cutáneas úlcerovegetantes de la blastomicosis sudamericana.

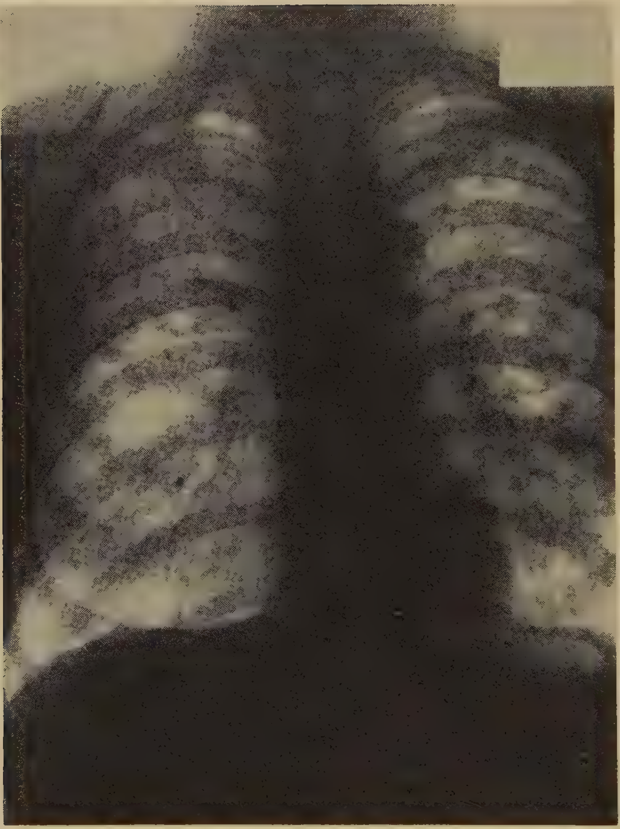

Fig. 3 – Lesiones pulmonares por diseminación hematógena (forma nodular agrupada) de la blastomicosis sudamericana.

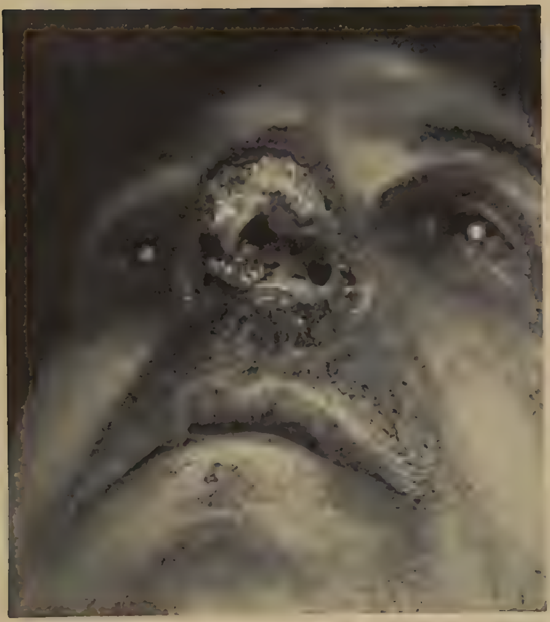

Fig. 4. – Forma cutáneomucosa de la histoplasmosis.

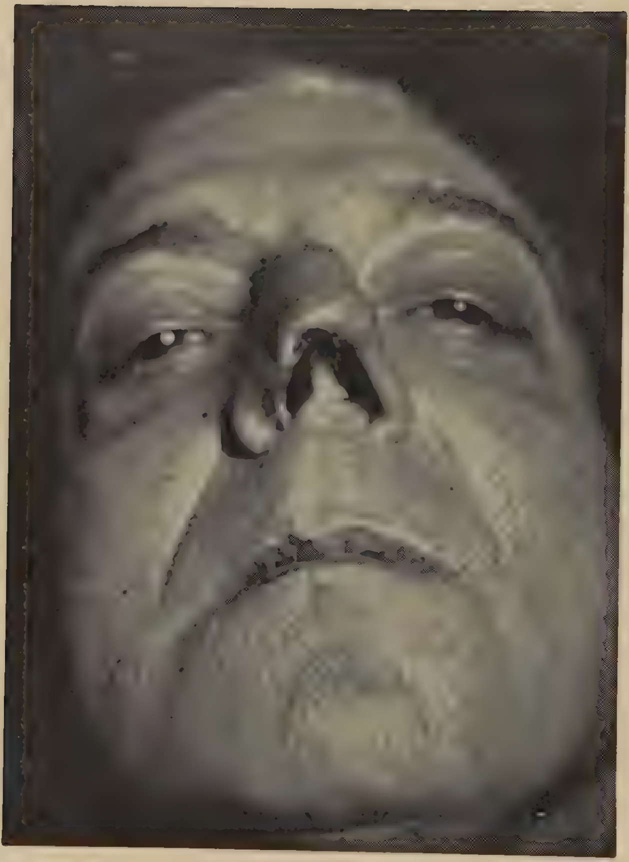

Fig. 5. – El mismo paciente curado con sulfamidoterapia.

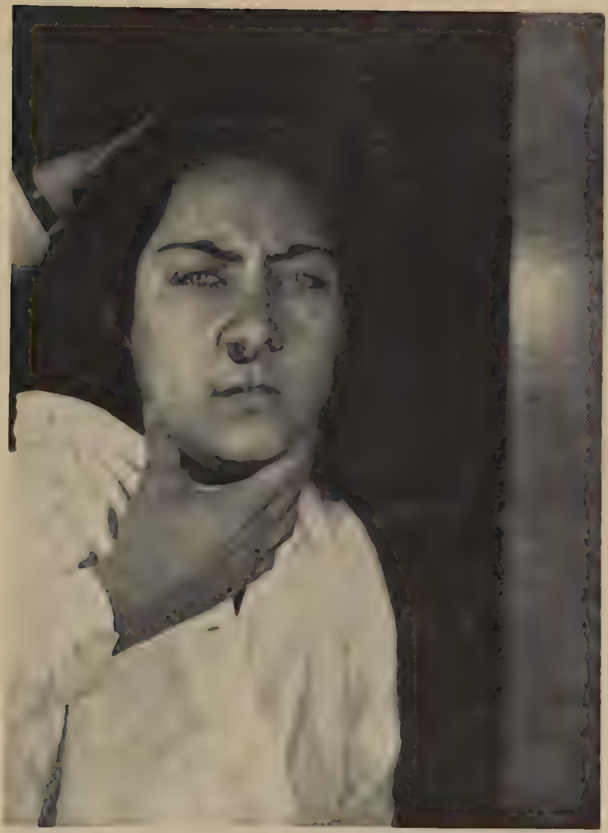

Fig. 6 – Lesiones úlcerovegetantes periorificiales en un caso de coccidioidomicosis con diseminación hemática.

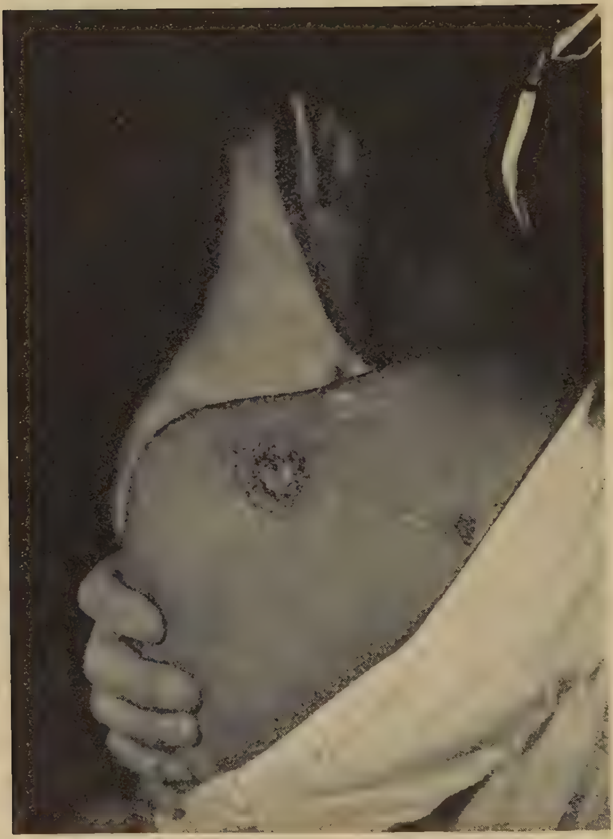

Fig. 7. – Lesión úlcerovegetante en el hombro de la misma enferma.

densamente infectada es el Chaco, siguiéndole, luego, en orden decreciente, Misiones, norte de Corrientes este de Tucuman y noreste de Santiago del Estero.

Las primeras manifestaciones aparecen frecuentemente a nivel de la mucosa bucofaringea. La siembra pulmonar por diseminación hematógena es bastante común, con poca sintomatología clínica y grandes alteraciones radiológicas. En el Centro de Micología de Buenos Aires, hemos visto un promedio de cinco casos por año en el último quinquenio.

Blastomicosis norteamericana: No se conocen casos autóctonos en la Argentina.

Esporotricosis: se presenta en forma esporádica siempre producida por el *Sporotrichum schenckii*, siendo la forma linfangítica localizada la más común.

Histoplasmosis: Se conocen unos veinte y cinco casos con manifestaciones clínicas. Estas consisten frecuentemente en lesiones ulcerosas de la mucosa bucal, faringea, laringea o nasal acompañadas de adenopatía satélite, como si de tratara de un complejo primario. En un caso las primeras manifestaciones fueron las de una pleuresia serofibrinosa y en otros las de la enfermedad de Hodgkin. La mayoria de los casos se ha registrado en la provincia de Buenos Aires al oeste de la Capital Federal y algunos en Córdoba.

No existe evidencia, hasta el presente, de la incidencia de formas asintomáticas, de primoinfección pulmonar ni de secuelas consistentes en focos calcificados en el pulmón o en otros órganos. Muchos de los casos han curado con el tratamiento sulfamídico (sulfadiazina) La histoplasmosis no constituye en la actualidad un problema sanitario de importancia en la Argentina. P. NEGROMI, F. NIÑO y colaboradores y D. PIERINI hicieron aportes sobre esta enfermedad.

Coccidioidomicosis: Existe en forma endémica en una zona árida paracordillerana que se extiende desde el paralelo 27,5° al 40° de latitud sud. Se han registrado formas evolutivas en Tucumán, Santiago del Estero Córdoba, Mendoza y Rio Negro. La incidencia de reactores positivos a la coccidioidina en niños de escuelas primarias rurales oscila de 18 a 27 %. Tampoco constituye un problema sanitario en la actualidad, puesto que tan solo se conocen unos quince casos evolutivos. La irrigación artificial del suelo y su cultivo son las medidas profilácticas que aconsejamos.

Nuestros estudios esperimentales han permitido reconocer la existencia del esporangio de primoinfección provisto de ostiolo y de protoplasma residual formando parte del ciclo evolutivo del parásito, publicado en 1950.

Trichosporosis: Se conocen hasta el presente dos casos clínicos con manifestaciones pulmonares seudotuberculosas, ambos de la provincia de San Juan.

Los agentes fueron clasificados como *Trichosporon cutaneum* y *fermentans* respectivamente.

Finalmente los casos de moniliasis pulmonares son relativamente

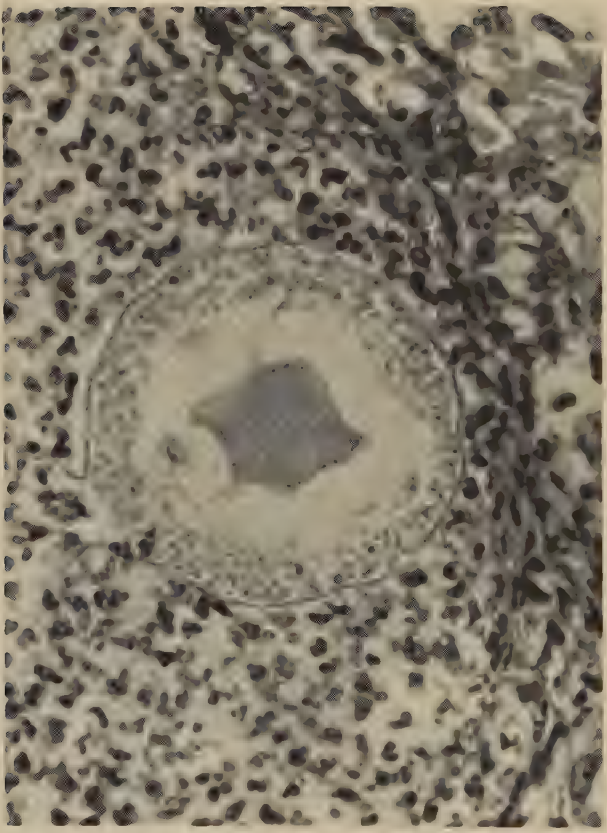

Fig. 8 – Esporangio con ostiolo y protoplasma residual (esporangio de primoinfección) del *Coccidioides immitis*.

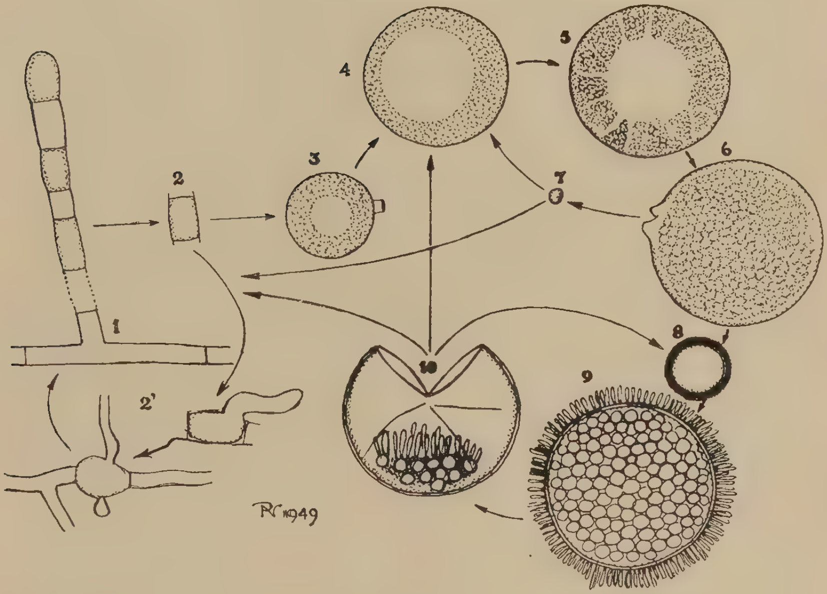

Fig. 9. – Ciclo evolutivo del *C. immitis*: 1, proconidia con entosporos; 2 entosporos; 1, 2 y 2', ciclo saprofítico. 3, 4, 5 y 6 esporangios de primoinfección (ciclo que se produce en la primera semana de la infección). 8, 9 y 10 formas quísticas con areola o formaciones radiadas acidófilas (ciclo que se produce despúes de la primera semana de la infección.

frecuentes, pero tan solo algunos han sido cuadros primitivos y que mejoraron o curaron con el tratamiento adecuado. Clínicamente se caracterizan por una bronquitis crónica con episodios agudos congestivos o de condensación pulmonar, por su tendencia hemoptoica, el buen estado general, ausencia de fiebre o de pequeña intensidad. Las radiografias acusan un aumento de la sombra parahiliar. No de conocen otras localizaciones internas de *Candida albicans* y, en cuanto a las formas generalizadas del lactante, están relacionadas con alteraciones del estado general, de su nutrición o a la hipovitaminosis.

En el mes de Octubre de 1956 tuvo lugar en la Argentina, un ,,Symposium'' sobre Moniliasis en el cual se presentaron numerosos trabajos divididos en las siguientes secciones: Pediatria, Dermatología, Medicina interna, Tocoginecología y Biología, y se publicarán en la Revista de la Asociación Médica Argentina.

Otros tres libros de Micología médica vieron la luz en estos últimos años. En 1952 el de los Drs LUIS C. VERNA y F. J. HERRERO publicado por el Ateneo de Buenos Aires. Contiene 740 páginas con 286 figuras y está dividido en 16 cápitulos con índice alfabético, glosario y bibliografía al final de la obra.

En 1953 el Dr. R. C. ZAPATER dió a conocer su obra Micología alergógena, editada igualmente por El Ateneo, consta de 210 paginas con 96 figuras. Está dividida en 15 capítulos y contiene un índice alfabético al final.

Finalmente del Dr. P. RUBINSTEIN publicó en 1954 un volumen sobre Micosis broncopulmonares (Editorial Beta) de 443 páginas y 101 figuras dividido en 10 capítulos con bibliografía, indice alfabético de autores y de materia.

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AUGUSTE SARTORY
(1881—1950)

IN MEMORIAM

AUGUSTE SARTORY

(1881—1950)

Le Doyen AUGUSTE SARTORY est né 22 mai 1881 à Limoges (France). En 1900, brillant étudiant, AUGUSTE SARTORY, obtenait à l'Ecole Supérieure de Pharmacie et à la Sorbonne, en se jouant, ses titres de pharmacien, de Licencié ès-sciences, de Docteur ès-sciences naturelles. Jeune lauréat, il est en 1904 préparateur (assistant) de Cryptogamie à la Faculté de Pharmacie de Paris ou il collabore avec les plus illustres chercheurs de ce temps: les Professeurs PORTIER, ROGER, CHIRAY, le Doyen RADAIS et bien d'autres.

En 1913, il est nommé chargé de cours de Pharmacie Chimique à l'Ecole Supérieure de Pharmacie de Nancy, après avoir obtenu les titres de Lauréat de l'Institut (Académie de Sciences), Professeur Agrégé de l'Ecole Supérieure de Pharmacie de Paris et Lauréat de l'Académie de Médecine par les prix Clarens, Osiris et Vernois.

En 1914, il a été chargé des cours d'Hydrologie et de Microbiologie. La seconde partie de ce dernier cours était destinée à l'étude des principaux parasites végétaux de l'homme, des animaux et des plantes. Il écrit lui-même à ce moment: ,,Née dans la première moitié du siècle dernier, avec GRUBY, l'étude des champignons pathogènes pour l'homme a pris dans ces dernières années, et sous l'influence de l'école pasteurienne, un développement qui lui assigne désormais une place importante parmi les diverses branches des sciences médicales et pharmaceutiques. Je donne donc une notion générale principale sur les mycoses et les champignons parasites que chaque scientifique ou pharmacien doit savoir rechercher, identifier et combattre."

En 1918, A. SARTORY opte pour le retour dans le pays de ses aïeux, l'Alsace, et en 1919 il est nommé professeur de Bactériologie et de Cryptogamie à la Faculté de Pharmacie de Strasbourg. En 1936, il devient Doyen de cette même Faculté; en même temps, il préside aux destinées de nombreuses sociétés alsaciennes: Société de Mycologie, Société Philomatique, etc.. A ce moment, il est élevé à la dignité de Commandeur dans l'Ordre de la Légion d'Honneur.

En 1939, il dirige l'évacuation de sa Faculté vers Clermont-Ferrand et a été mis à la retraite forcée par le gouvernement de Vichy en 1943. En 1945, il ramène sa Faculté et ses étudiants à Strasbourg où il réorganise les Laboratoires de son Institut.

En 1947, A. SARTORY est appelé aux fonctions de Professeur de Microbiologie et de Cryptogamie à la Faculté de Pharmacie de Paris.

Le 2 décembre 1950, A. SARTORY succombait, terrassé par l'épuisement provoqué par ses multiples occupations scientifiques et administratives.

Au point de vue de son oeuvre scientifique, sa première communication datant de 1906 est intitulée ,,Sur l'existence constante d'une Levure chromogène dans les sucs gastriques hyper-acides''. Cette Levure est le *Cryptococcus salmoneus*, retrouvé plus tard par BEAUVERIE et LESUEUR de Lyon.

En 1908, A. SARTORY soutient une thèse de Doctorat ès-sciences naturelles intitulée: ,,Etude expérimentale de l'influence de l'agitation sur les champignons inférieurs''. Il y rend compte pour la première fois de l'exaltation des propriétés biologiques de ces organismes par l'agitation. On sait maintenant que l'agitation du milieu revêt une importance majeure dans la genèse des anticorps au cours du développement de certains Cryptogames inférieurs.

Les publications de A. SARTORY se succèdent régulièrement de 1907 à 1911, et toutes ont trait aux champignons inférieurs. En 1907, il publie avec le Professeur CHIRAY une étude sur le muguet intestinal chez les nouveau-nés; avec DEMANCHE, il étudie une Levure, *Cryptococcus regerii*, isolée d'un pus de péritonite par perforation de l'estomac. En 1908, avec le Professeur CLERC, il fait l'étude approfondie d'une Levure de la gorge, provoquant une angine chronique et celle d'un Bacille rouge trouvé dans une Algue nommée *Codium busea*. La même année, avec BAINIER, l'éminent mycologue français, dont il fut l'élève, il découvrit l'*Aspergillus fumigatoides*, qui donne facilement des asques et des ascospores. Rappelons encore les travaux avec PORTIER sur une bactérie commensale d'un *Isaria*, en 1911; avec BAINIER, en 1912, il étudie cinq *Citromyces* nouveaux et plusieurs *Aspergillus* (voir THOM — Manual of Aspergilli). Dans la même année il décrit l'étude biologique et morphologique d'un certain nombre d'*Aspergillus* (*schellei*, *ombrosus*, *pénicillium*). Il étudie différents agents pathogènes tels que *Penicillium olsoni*, *Trichoderma varians*, *Fusoma intermédia* et surtout une Levure du genre Willia, qui produit des formes sexuées sous l'influence d'une bactérie, isolée d'une lésion pathologique. Les empoisonnements par les champignons supérieurs, particulièrement de *Entoloma lividum*, ont attiré son attention.

En 1913, il fait l'étude, avec BAINIER, d'un champignon nouveau du genre Gymnoascus: *G. confluens*; avec ORTICONI, il étudie un Sporotrichum provenant d'une sporotrichose d'un métacarpien. Puis il décrit *Sterigmatocystis sydowi*, puis différents Penicilliums, Diplocladium, des Rhizopus et Muratella.

En 1914 il décrit l'étude biologique d'un Cryptococcus et d'une Levure rose, puis, avec ROGER et MENARD il donne les détails sur une nouvelle mycose, la Chalarose. Son attention est attirée vers les organismes de la fermentation du lait et la flore cryptogamique dans les mines de fer.

La question fondamentale de l'immunité ne laisse pas A. SARTORY indifférent. Dès 1908, avec Chiray, il publie un mémoire sur l'imperméabilité rénale aux agglutinines et sensibilisatrices typhiques; avec Radais en 1910, il étudie l'immunité du lapin contre le poison des Ammanites à phallines. Il est chargé par les Services ministériels de l'Hygiène d'étudier l'action nuisible des poussières organisées dans l'industrie. A la même époque il met au point un procédé de stérilisation de l'air au moyen d'un appareil muni d'une résistance électrique.

Durant la période de la guerre 1914—1918, A. Sartory publie un certain nombre de notes dans les différentes Académies, qui concernent toute la Microbiologie et ont surtout trait à la gangrène gazeuse, aux paratyphoïdes et au tétanos.

A partir de 1919, à Strasbourg, A. Sartory, en collaboration avec son frère et J. Meyer, continue ses recherches sur le terrain de la mycologie et de la microbiologie en général. Nous citons en première ligne les travaux comprenant l'étude de l'action du Radium sur l'*Aspergillus fumigatus* en culture sur milieu dissocié et non-dissocié, l'apparition sous cette influence des périthèces qui n'avaient pas été mis en évidence jusqu'ici. Ces travaux ont donné lieu à la publication d'importants mémoires.

Puis viennent en 1928 l'étude de différents cas d'onycomycoses (Trichophyton, Hemispora, différentes Levures), ainsi que l'étude des mycoses osseuses. Ces travaux ont surtout été élaborés en collaboration avec J. Meyer, M. Meyer, Allenbach et Zimmer. Ces travaux ont défini les méthodes de diagnostic et de traitement de ces mycoses osseuses.

En 1933, la description du cycle évolutif des Actynomyces dans les cultures et in vivo, après passage à travers l'ultrafiltre au collodion, a été donnée en collaboration avec J. Meyer et Antonioli. En 1936, il étudie un cas d'actinomycose primaire vertébrale, en collaboration avec J. Weil et J. Meyer.

Jusqu'en 1941, l'étude de ces actinomycoses préoccupe principalement son école et plusieurs Actinomyces acido-alcoolo-résistants ont pu être isolés à partir de lésions pulmonaires, rénales et laryngées. En 1943, avec Wurtz, il réalise la production de périthèces de *Sterigmatocystis nigra*, grâce aux effets du radium. En dernier lieu, à partir de 1945, A. Sartory s'intéresse aux Antibiotiques des végétaux supérieurs, parmi lesquels il étudie, avec J. Meyer, principalement un facteur bactériostatique de la tomate fraîche.

Les travaux de A. Sartory ont ainsi été presque totalement orientés vers la pathogénèse des affections mycosiques, mal connues ou inconnues. Dans ce domaine, il a introduit une évidente clarté. Il est l'auteur, avec ses collaborateurs, d'un traité sur les *champignons parasites de l'homme et des animaux*, auquel, depuis sa parution, cinq suppléments ont été ajoutés, le dernier en 1949. A cette époque il préside toujours aux destinées de la Société Strabourgeoise de Mycologie. Avec René Sartory et J. Meyer, il publie en 1950 un important Traité de Microbiologie Pratique. Ce volume de 1000 pages, orné de nombreuses planches en couleurs, a trouvé un accueil très favorable dans les laboratoires de microbiologie et chez les étudiants en médecine et en pharmacie.

On voit que l'oeuvre scientifique que laisse A. SARTORY, Membre titulaire de l'Académie de Pharmacie et Membre correspondant de l'Académie de Médecine, est très importante et ne saurait être ignorée par tout mycologue.

Nous souhaitons que l'exemple de sa persévérante activité reste présent à la pensée de nombreuses générations de scientifiques fréquentant les laboratoires de microbiologie et de cryptogamie pathologique.

JACQUES MEYER

Faculté de Pharmacie
Strasbourg, France

FRED WILBUR TANNER
(1888—1957)

IN MEMORIAM

FRED WILBUR TANNER

Professor Fred Wilbur Tanner died Sunday February 24, 1957 at his home in Winter Park, Florida after a prolonged illness. He had been on leave of absence from the department since September 1948 because of ill health.

His early training was at Wesleyan University in Middletown, Connecticut. Here he received his bachelor's degree. His advanced training was taken at the University of Illinois where he obtained his master's degree in 1914 and his doctor's degree in 1916. Because of his outstanding contributions to the field of Public Health, Wesleyan College awarded him an honorary Doctor of Science degree in 1943.

Prior to an appointment to the faculty of the University of Illinois in 1915 he held the position of Bacteriologist in the Illinois State Water Survey. He served on the staff of the Department of Bacteriology from 1915 until 1921 when he was made head of the department. He served in this capacity until 1948 when illness forced him to retire. He was the first head of the department and it was under his capable leadership that the department developed.

Tanner's early interest was in yeasts. The Tanner Collection of yeast cultures was a part of the American Type Culture Collection, and at one time was probably the largest in the country. His interest in this group of microorganisms resulted in a translation from the French of Guillerimond's ,,The Yeasts'', which was a tremendous aid to American scholars in this field of work.

Throughout his career he was interested in Food Bacteriology, a field in which his major research was accomplished. The many outstanding contributions established him as an authority in this field, not only in this country but in foreign lands as well. He was the author of several texts. A beginning text entitled Bacteriology and an accompanying laboratory manual ran through four editions. The Microbiology of Foods and Food Borne Infections and Intoxications were used as reference texts in many universities. The latter book was revised after he was forced to leave the department.

In 1952 Professor Tanner was awarded the Stephen M. Babcock award by the Institute of Food Technologists for outstanding contributions to food technology. He had previously served as President of the Institute and was Editor in Chief of Food Research a periodical devoted to reports of original research relating to problems in the food field. He was active in the Society of American Bacteriologists and assisted in establishing the Society of Illinois Bacteriologists, a branch of the national organization. He was elected the first president of the Illinois group and served in this capacity for two years.

Not all of his interests were confined to the University and his academic career. He was a leader in church and civic affairs in the Urbana-Champaign community. He was an active member of Trinity Methodist Church, a past president of the Urbana Rotary Club and an active participant in many volunteer civic enterprises.

Department of Bacteriology F. M. Clark
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Publications of F. W. Tanner

1928-1929

Book notices:

J. H. Dibble, Recent advances in bacteriology and the study of the infections. *J. Parasitol.* **15**: 225. (300)

Articles:

with Bollas, G. D.

Action of several disinfectants on yeasts. *Proc. Soc. for Exper. Biol. and Med.* **25**: 154—56. (750)

with Elder, A. L.

Action of bacteriophage on psychrophillic organisms. *J. Infect. Dis.* **43**: 403—06. (1800).

with Black, L. A.

A study of thermophilic bacteria from the intestinal tract. *Cent. Bakt.* II. **75**: 360—75. (6000)

with Wallace, G. I.

Studies on the "Bios question." *Ibid.* II. **76**:1:1—17. (7000)

Books:

Bacteriology: A text-book of microorganisms. John Wiley and Sons, Inc., N. Y. 1928. 548 pp.

Practical bacteriology—an introduction to bacteriological technic. John Wiley and Sons, Inc., N. Y. 1928. 234 pp.

1929-1930

Articles:

Yeasts. The Encyc. Brit. 14th Ed. **23**:881. (600)

with Windsor, Mary F.

The ability of Escherichia coli and Serratia marcescens to survive 62.8° C. for thirty minutes in milk. *J. Dairy Sci.* **12** (1929) : 202—10. (3500)

with Wallace, G. J.

The standardization of disinfectants. *Cent Bakt.* I. **114** (1929) : 161—74. (4600)

1930-1931

Book notices:

Molds, yeasts, and actinomycetes—a handbook for students of bacteriology. *Arch. Surg.* 1930.

Articles:

With H. Green:

Residual germicidal action of water and plain agar after exposure to ultraviolet light *Proc. Soc. for Exp. Biol. and Med.* **28:** 291—93. (1080)

With William Burrows:

The viability of yeasts in sucrose solutions. *J. Bact.* **21:** 32 (250)

With G. I. Wallace:

The effect of salt and sugar solutions on the thermo death times of moids. *Ibid.* **21:** 1:32. (300)

1931-1932

Articles:

With Roy Hansen:

The nodule bacteria of the leguminosae with special reference to the mechanism of inoculation. *Cent. Bakt.* II. **85:** 129—52. (9600)

With G. I. Wallace:

Effect of freezing on microorganisms in various menstra. *Proc. Soc. Exp. Biol. and Med.* **29:** 32—34. (680)

Effect of heat on mold spores. *Ibid.* **28:** 970—72. (1100)

1932-1933

Books:

The microbiology of foods. Twin City Printing Co., Champaign, Ill. 1932 776 pp.

Articles:

With E. E. Echelberger and F. M. Clark:

"Autosterilization" as a problem in the bacteriological examination of canned foods. *Proc. Soc. Exp. Biol. Med.,* **29:** 1000—01 (510)

With E. H. Ruyle:

Penetration of intestinal wall of rabbits by yeasts. *Ibid.,* **29:** 1001—03. (960)

With Grace B. Armstrong:

Home canning of foods for family use. *Ill. Agr. Exp. Sta. Circ.* **394.** (8000)

With Florence Evans:

Effect of meat curing solutions on anaerobic bacteria. I. Sodium chloride. *Zent Bakt,* II: **88:** 44—54. (4900)

1933-1934

Books:

Food-borne infections and intoxications Twin City Printing Co., Champaign, Ill., 1933. VIII + 439 pp.

Articles:

Food intoxication and infection. *J. Amer. Diet. Assoc.,* **9:** 279—85. (2400)

With Florence L. Evans:

Effect of meat curing solutions on anaerobic bacteria. II. Sodium nitrate. *Zent Bakt.* **89:** 48—54. (4900)

See also G. I. Wallace.

1934-1935

Articles:

Some bacteriological problems involved in home canning procedures. *J. Home Econ.,* **26:** 365—76. (5000)

Home canning and public health. *Amer J. Pub. Health,* **25:** 301—13. (7700)

The microbiological examination of fresh and frozen fruits and vegetables. *Ibid.,* **24:** 485—92. (5000)

With J. R. Byerley:

The effect of ultraviolet light on the fermenting ability of yeasts. *Arch. für Mikro.,* **5:** 3:349—57. (3500). Abstract in *Trans. Ill. Acad. Sci.,* **27:** 65. (500)

With Grace B. Armstrong:
Home canning of foods for family use. *Ill. Agr. Exp. Sta. Circ.* 394. (Rev. ed.) (10.500

With Florence L. Evans:
Effect of meat curing solutions on anaerobic Bacteria III. Sodium nitrite. *Zent. Bakt.*, Abt. 2. (4500)

Effect of meat curing solutions on anaerobic bacteria. IV. The effect of mixed curing solutions *Ibid.* (3500)

With Doris L. Schneider:
Effect of temperature of storage on bacteria in water samples. *Proc. Soc. Exp. Biol. Med.* (2500)

1935-1936

Articles:

Proper processes for home canners. *J. Amer. Diet. Assoc.*, **11:** 1: 18—27. (4320)
Poisonous substances in food (discussion). *Amer. J. Pub. Health*, **26:** 385—86. (1340)
Public health significance of sewage sludge when used as a fertilizer. *Sewage Works J.*, **7:** 4: 611—17. (2450)

With Viola Michael:
Microbiology of merchantable cream-style canned corn. *Foods Res.*, **1:** 99–112. (4800

1936-1937

The microbiology of canned foods. *Proc. 2d Internat. Cong. Microbiol.* 1936, pp. 219.20 (1000)
Report on sampling inoculum *J. Assoc. Official Chem.*, Aug., 1936, 431—33. (800)
Microbiology of fresh and canned foods. *Food Mfr.*, **11:** 385—89. (3600)
Some recent advances in food poisoning. *N. Amer. Veterinarian*, **18:** 19—23. (2700)

With Elaine W. Oglesby:
Influence of temperature on growth and toxin production by Clostridium botulinum *Food Res.*, **2:** 481—94. (3900)

With F. M. Clark:
Thermophilic canned-food spoilage organisms in sugar and starch. *Ibid.*, **2:** 27—40. (4000)

1937-1938

Books:

Bacteriology: a textbook of microorganisms (3d ed.). John Wiley and Sons, N.Y., 1937. 510 pp.

Articles:

With S. C. Prescott:
Microbiology in relation to food preservation. *Food Res.*, **3:** 189—97. (4000)

With J. Hofer:
Thermal death-time studies of Oospora lactis. *Ibid.*, **2:** 505—13. (3200)

With P. R. Beamer:
Presence of acid-fast organism on tableware. *Zent. f. Bakt.*, **2:** 98: 209—12 (1300)

With E. Wheaton and R. H. Lueck:
Observations on problems relating to the paper milk bottle. *J. Milk Tech.*, **1:** 11—15 (3000)

1939-1939

Articles:

The present status of the paper milk container. *J. Milk Technol.*, **2:** 4—15. (10.000)
Microbial flora of paper containers. *Amer. J. Pub. Health*, **28:** 587—92. (3100)
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Bacteriology in high school biology. *Sci. Teacher*, **6:** 1—6. (3800)

With P. R. Beamer:

Resistance of non-spore-forming bacteria to heat *Zent. f. Bakt.*, **2:** 100: 81—98. (8600)

Heat resistance studies on selected yeasts. *Ibid.*, **2:** 100: 202—11. (3400)

With F. M. Clark:

Studies on the bacterial metabolism of sulfur. III. Formation of hydrogen sulfide by thermophilic bacteria. *Ibid.*, **2:** 98: 298—311. (5000)

With Georgia Pitner:

Germicidal action of bromine. *Proc. Soc. Exp. Biol. Med.*, **40:** 143—45. (500)

1939-1940

Articles:

With E. Wheaton and C. O. Ball:

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With H. F. Lewis:

Germicidal properties of hot paraffin. *Food Ind.*, Mar.-Apr., 1940, pp. 57—58; *Oil and Soap*, **17:** 26—30. (5600)

With P. R. Beamer and C. J. Rickher:

Further observations on influence of temperature on growth of and toxin formation by *Clostridium botulinum*. *Proc. 3d Internat. Cong. Microbiol.*, Sept. 1939, pp 700—01. (700)

1940-1941

Articles:

With C. W. Houston:

Survival of microorganisms in physiological sodium chloride solution and distilled water. *Zent. Bakt., Abt.*, **102:** 353—60. (3500)

With P. M. Snyder:

Bacteriology of candy. *Proc. 1st Food Conf.*, Instit. Food Technologists, pp. 307—21. (4000)

1941-1942

Articles:

With J. W. Appling:

Bactericidal effect of ultraviolet rays on non-spore forming bacteria and mold spores. *Proc. Soc. Exp. Biol. Med.*, **47:** 47—51. (2000)

Bactericidal effect of ultraviolet rays on microorganisms on restaurant glassware. *Ibid.*, **47:** 51—54. (1500)

1942-1943

With Fren L. Wilson:

Germicidal action of aliphatic alcohols. *Proc. Soc. Exp. Biol. Med.*, **52:** 138—40 (700

With J. Forgacs:

Longevity of pathogenic bacteria in apple juice. *Fruit Products J.*, **22:** 10: 295—99. (8500)

1943-1944

Books:

The microbiology of foods (2d ed.). Garrard Press, Champaign, Ill. 1206 pp.

Articles:

With J. Forgacs:

Longevity of pathogenic bacteria in apple juice. *Fruit Prod. J.*, **22:** 295. (7000)

1944-1945

Books:

The microbiology of foods (2d ed.). Garrard Press, Champaign, Ill., 1944. 1204 pp.

Articles:

Sanitary care and handling of foods. *J. Amer. Diet. Assoc.*, **21:** 137—41. (6000)

With Ferne Wilson:

Behavior of pathogenic bacteria in fermented milks. *Food Res.*, **10:** 122—34. (7000)

With *J.* Forgacs and W. A. Ruth:

Freezing of apple juice. *Food Res.*, **10:** 148—59. (7500)

1945-1946

Articles:

With J. M. Severens:

The inheritance of environmentally induced characters in bacteria. *J. Bact.*, **49:** 383—93. (4300)

With *M. T.* Jones:

Incidence and distribution of Clostridium botulinum in soils of Illinois *Food Res.*, **10:** 238—45.

With A. T. Webb:

Effect of spices and flavoring materials on growth of yeasts. *Ibid.*, **10:** 273—82. (6400)

1946-1947

Articles:

New aspects of the food poisoning problem. *J. Amer. Dietetic Assn.*, **23:** 16—21.

1947-1948

Books:

With Louise P. Tanner:

American Journal of Public Health, 35 year index, vols. 1-35, 1911-1945. Garrard Press, Champaign, 1948.

With F. W. Tanner, Jr.:

Bacteriology—a text book of micro-organisms (4th ed.). John Wiley & Sons, Inc., N.Y., 1948.

Articles:

With Dorothy Husseman:

Relation of certain cooking procedures to Staphylococcus food poisoning. *Amer. J. Pub. Health*, **37:** 1407—14.

With A. J. Shanahan:

Further studies on the morphology of Escherichia coli exposed to penicillin. *J. Bact.*, **55:** 537—44.

With A. J. Shanahan and A. Eisenstark:

Morphology of Escherichia coli exposed to penicillin as observed with the electron microscope. *Ibid.*, **54:** 183—89.

1948-1949

Articles:

Paperboard and paper food containers—analysis of literature and appraisal of their sanitary condition. *J. Milk & Food Tech.*, **11:** 334—45.

Paper and paper board in the food industry—public health aspects. *Amer. J. Pub. Health*, **38:** 1688—93.

With Dorothy Hussemann:

A comparison of strains of staphylococci isolated from foods. *Food Res.*, **14:** 91—97.

RHODA W. BENHAM
1894—1957

IN MEMORIAM

RHODA W. BENHAM (1894—1957)

RHODA W. BENHAM was one of the foremost research scientists and teachers in the field of medical mycology. Her many publications on the morphology and physiology of the fungi that are medically important have become an important part of the body of literature on this subject, and have served to stimulate research in many phases of mycology. Her laboratory served as a training ground for many students who have since become active workers both here and abroad. Her careful work and her enthusiasm for every new finding or useful technique has been a continued inspiration to mycologists all over the world.

This outstanding woman was born in Cedarhurst, Long Island, on December 5, 1894, and grew up in the environs of New York City. She attended Barnard College, where her chief interest was botany, and received the A. B. degree in 1917 and the M. A. degree in 1919. At the time she received the latter she was assistant in the Botany Department of the school, a position she retained until 1925. She received her Ph. D. from Columbia University in 1931, and in 1943 the school named her Associate Professor of Dermatology, a position she retained until her failing health forced her to retire in 1956.

In 1926, Dr. BENHAM began her long association with the Department of Dermatology at Vanderbilt Clinic. There with the late J. GARDNER HOPKINS, Chief of the Department of Dermatology, she provided the Clinic with diagnostic service in mycology. She also helped to establish and develop a research laboratory for the study and teaching of medical mycology at Columbia Medical School. From 1948 to 1953 she was consultant in mycology to the New York State Department of Public Health, however, in connection with her work she received clinical materials and culture specimens from all over the world.

Dr. BENHAM's research was particularly concerned with the yeast-like fungi, and her fundamental studies with the *Candida* and *Cryptococcus* species and with *Pityrosporum ovale* are well known by workers in medical mycology. In recent years she published a number of papers on the dermatophytes, particularly in regard to the effect of nutrition on the morphology of these organisms. Her exhibits won prizes on several occasions at the annual conventions of the American Medical Association, and a gold medal at the 9th International Dermatological Congress held in Budapest in 1935.

This eminent mycologist held membership in a number of honorary and scientific organizations including: Society of The Sigma Xi, Harvey Society, American Association for the Advancement of Science, Mycological Society of America, New York Academy of Sciences, and the Microbiology Section of the New York Academy of Medicine.

On her birthday in 1956, former students and associates honored Dr. Benham by presenting her with a book of letters and photographs reminiscent of her career. She died January 18, 1957.

Mycology Unit
Communicable Disease Center
Chamblee, Georgia

Lucille K. Georg

PUBLICATIONS BY Dr. Rhoda W. BENHAM

1. Monilia infections of the hands and feet. New York State J. Med. **29:** 793—800, 1929 (with J. G. Hopkins).
2. Asthma due to a fungus, Alternaria. JAMA **94:** 6—10, 1930 (with J. G. Hopkins and B. M. Kesten).
3. Sensitization to saprophytic fungi in a case of eczema. Proc. Soc. Exp. Biol. Med. **27:** 342—344, 1930 (with J. G. Hopkins and B. M. Kesten).
4. Certain monilias parasitic on man; their identification by morphology and by agglutination. J. Infect. Dis. **49:** 183—215. 1931.
5. Phoma condiogena, an excitant in asthma: some observations on the development and cultural characteristics. Bull. Torrey Bot. Club, **58:** 203—214, 1931.
6. Monilias, yeasts and cryptococci; their pathogenicity, classification and identification. Am. J. Pub. Health, **22:** 502—504, 1932.
7. Sporotrichosis in New York State. N.Y. State Jour. Med., **32:** 595—601, 1932 (with J. G. Hopkins).
8. Sporotrichosis: its transmission to plants and animals. J. Infect. Dis., **50:** 437—458, 1932 (with B. M. Kesten).
9. Fungus infections of the skin and its appendages occurring in Puerto Rico; a clinical and mycologic study. Arch. Derm. Syph. **25:** 1046—1057, 1932 (with B. M. Kesten, B. K. Ashford, C. W. Emmons and M. C. Moss).
10. Vegetable parasites that attack both plant and human life. J. New York Botanical Garden, **33:** 149—153, 1932.
11. Yeast-like fungi found on the skin and in the intestines of normal subjects: a survey of 100 persons. Arch. Derm. Syph. **28:** 532—543, 1933 (with A. MCH. Hopkins).
12. The fungi of blastomycosis and coccidioidal granuloma. Arch. Derm. & Syph., **30:** 385—400, 1934.
13. The terminology of the cryptococci with a note on Cryptococcus mollis. Mycologia, **27:** 496—502, 1935.
14. Cryptococci — their identification by morphology and by serology. Jour. Infect. Dis., **57:** 255—274, 1935.
15. Pathogenic Fungi; chapter 45 in "Agents of Disease & Host Resistance", C.C. Thomas, Springfield, 1935.
16. Experimental studies with the dermatophytes. 1. Primary disease in laboratory animals. J. Invest. Derm., **1:** 451—467, 1938, (with E. D. De Lamater).
17. Experimental studies with the dermatophytes. 2. Immunity and hypersensitivity produced in laboratory animals. J. Invest. Derm., **1:** 469—488, 1938 (with E. D. De Lamater).
18. The fungi causing deep-seated infections; their diagnostic characteristics and classification. Proc. 6th Pacific Science Congress, **5:** 863—872, 1939.
19. The cultural characteristics of Pityrosporum ovale — a lipophylic fungus. J. Invest. Derm. 187—203, 1939.

20. Cultural characteristics of Pityrosporum ovale — a lipophylic fungus. Nutrient and growth requirements. Proc. Soc. Exp. Bio. & Med. **46: 176—178, 1941.**
21. The nutrient and growth requirements of Pityrosporum ovale — a lipophylic fungus. (Abstract) Torreya **41:** 68—69, 1941.
22. Laboratory procedures in the diagnosis of fungus diseases. Proc. New York State Assoc. Pub. Health Labs. **24:** 69—82, 1944.
23. Pityrosporum ovale — a lipophilic fungus. Thiamin and oxaloacetic acid as growth factors. Proc. Soc. Exp. Bio. & Med. **58:** 199—201, 1945.
24. Biology of Pityrosporum ovale. Chapter 4 in Nickerson's "Biology of Pathogenic Fungi" Ann. Cryptogam. Phytopathol. VI, Chronica Botanica Pub. Co., Waltham, Mass. 1947.
25. Allescheria boydii, causative agent in a case of meningitis. J. Invest. Derm. **10:** 99—110, 1948 (with L. K. Georg).
26. Effect of nutrition on growth and morphology of the dermatophytes. 1. Development of macroconidia in Trichophyton rubrum, Mycologia, **40:** 232—240, 1948.
27. Pigment production in the differentiation of Tricophyton mentagrophytes and Trichophyton rubrum. Mycologia, **41:** 291—302, 1949 (with F. C. Bocobo).
28. Cryptococcosis and blastomycosis. Ann. New York Acad. Sciences, **50:** 1299—1314, 1950.
29. The Laboratory of Medical Mycology. The Biopsy, **2:** 1—2, 1950.
30. Nutritional studies of the dermatophytes with special reference to Trichophyton megnini Blanchard 1896 and Trichophyton gallinae (Megnin 1881), comb. nov. J. Invest. Derm. **18:** 453—472, 1952 (with M. Silva).
31. An unusual finding in Epidermophyton floccosum. J. Invest. Derm. **19:** 315—317, 1952 (with P. McCormack).
32. Nutritional studies of the dermatophytes — effect on growth and morphology, with special reference to the production of macroconidia. Trans. New York, Acad. Sciences, **15:** 102—106, 1953.
33. Maduromycosis of the central nervous system. Jour. Neuropath. & Exp. Neuro. **12:** 158—168, 1953 (with S. M. Aronson & A. Wolf).
34. The genus Beauveria, morphological and taxonomical studies of several species and of two strains isolated from wharf-piling borers. Mycologia, **45:** 727—746, 1953 (with J. L. Miranda).
35. Chemical analysis of the capsular substance of Cryptococcus neoformans, J. Invest. Derm. **22:** 279—283, 1954, (with J. M. Einbinder & C. T. Nelson).
36. Nutritional studies of the dermatophytes with special reference to the red-pigment producing varieties of Trichophyton mentagrophytes. Jour. Invest. Derm., **22:** 285—294, 1954 (with M. Silva).
37. Treatment of onychomycosis due to Trichophyton rubrum. A.M.A. Arch. Derm., **71:** 53—55, 1955 (with B. M. Kesten & M. Silva).
38. The genus Cryptococcus: the present status and criteria for the identification of species. Trans. New York Acad. Sciences, **17:** 418—429, 1955.
39. Cryptococcus neoformans: "an ascomycete". Proc. Soc. Exp. Bio. & Med. **89:** 243—245, 1955.
40. Trichophyton rubrum infections: a clinical, mycologic and experimental study. Jour. Invest. Derm. **25:** 311—328, 1955, (with B. M. Kesten and M. Silva).
41. The genus cryptococcus. Bact. Reviews, **20:** 189—200, 1956.
42. Species of Candida most frequently isolated from man: methods and criteria for their identification. J. Chronic Dis. 1957, (in press-posthumously).
43. The chlamydospores of Candida albicans: comparison of three media for their induction. J. Lab. & Clin. Med. 1957 (in press-posthumously).

LIBRI NOVI

Wuesthoff, Freda: *There is no time to be lost. Man in the Atomic Age.*

Otto Maier Verlag, Ravensburg, Germany, 1957, 21 pages.

The late Dr. Freda Wuesthoff was a physicist, a pupil of Professor Wien, the radiation scientist, and of Professor Sommerfeld, the theoretical physicist, who specialized in the atomic field. This is a posthumous edition of an address, presented in eight different languages in this volume. The author warns against the use of atomic energy for purposes of destruction and against the danger of radioactive contamination. The presentation of her ideals, characterized by deep sincerity and great humanity, leads into an utmost controversial field concerning the effect of atom splitting and atom fusion on the future of the whole human race. The biologist's only task can be to seek the truth by investigating the effect of radiation energy on living organisms. Mycologists have a great opportunity to help find this truth. The fallout's effect is too complicated to be understood by laymen, potentially too devastating to be ignored by them.

Tibor Benedek

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Gallery of Contemporary Noted Mycologists
8

REPORT OF A CASE OF BLACK GRAIN MADUROMYCOSIS OF THE FOOT CAUSED BY AN ABERRANT STRAIN OF MADURELLA MYCETOMI (LAVERAN) BRUMPT ON CURACAO, NETHERLANDS ANTILLES.

by

G. A. de Vries*), and Ludolph H. van der Hoeven**).

(25.IX.1957)

(With 3 fig.)

Case history

The patient, a 38 year old man, born at St. Lucia, British West Indies, and resident on Curaçao (Netherlands Antilles) since 1947, went to the doctor on May 16, 1953 complaining of a recent swelling on the right foot. According to the patient the swelling had started as a small nodule of pea-size between the bases of the first and second toe, being situated principally at the dorsal side of his foot about 2 months before he consulted the doctor. It had gradually increased in size and had become painful. On June 25, 1953 the patient was admitted to a hospital at Curaçao, the swelling being unchanged. June 26th surgical excision was performed. The pathological tissue was ill defined and adherent on the surrounding skin and deeper structures. The lesion was excised as completely as possible. The removed tissue had soft areas and discharged on pressure some pus and tiny dark brown granules.

Treatment

After excision of the tumor the patient was treated with streptomycin 1 gram every day over a period of 9 days. After his discharge from the hospital on July 7, 1953 he was X-rayed twice a week till August 31, 1953. The roentgenologist noticed good regression with 750 R. in 2 fields. On March 4, 1954 the man was without complaints, only a scar being visible on the place of the excision.

Pathology

Four pieces of formalin fixed tissue were received. They were of various size, measuring about ½ to 2 cm in diameter and of irregular form. They were composed of rather firm glistening white tissue with several yellow areas containing some pus and minute

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*) Mycologist, Baarn, The Netherlands.
**) Pathologist, Columbus, Ohio, U.S.A.

dark brown granules of about 1 mm diameter. Histological sections showed tissue composed chiefly of collagenous connective tissue with large areas of granulation tissue. These have in their central parts a scant amount of pus with large brown masses containing the fungus. The granulation tissue has many small vessels and is composed of fibroblasts, a great number of large vacuolated histiocytes, several giant cells of the foreign body type and an inflammatory infiltrate of mainly lymphocytes. The pus consists of debris and a few leukocytes and remnants of fibers. The large brown masses are sharply defined, round or more elongated or complicated and branching. In the unstained and in the hematoxylin and eosin stained preparations these masses were brown and showed a radiating pattern of hyphae. Outside of these brown masses no fungi could be demonstrated in the granulation tissue.

Mycology

Direct examination — The granules can already be seen under the low power of the microscope. Under high power lenses the hyphae show as empty tracks in the yellowish brown substance of the granules.

Staining techniques, like Gram's, Ziehl-Neelsen's and the periodic-

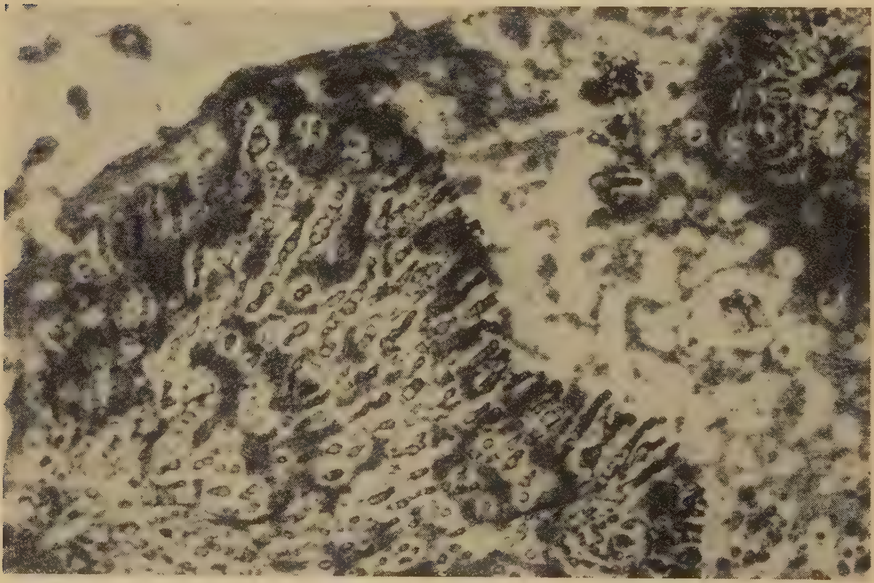

Fig. 1.

Brown granule from pus showing radiating pattern of fungus as seen in histological sections under high power, Silver stain.

acid-Schiff stain, show the hyphae and the granules more distinctly than the hematoxylin eosin method. The best results were obtained with the silver-impregnation methods, which are used for ,,staining" reticulin. At Curaçao one of the authors used the Gömöri technique and some of its variants. Although not only the fungus, but also other structures are impregnated, the diameter of the hyphae and the distribution of the granules can clearly be seen. This method has been used at Curaçao for several years in different cases of maduromycosis caused by different species and has always proved to give the best results in sections. It is, however, unsatisfactory for fungal elements in skin scales or for culture preparations, because the results are inferior and the method in itself is too elaborate. A possible explanation might be, that some substance is produced by the direct contact between fungus and host tissue, which is easily impregnated by the silver particles. The granules are composed of a yellowish brown interstitial substance in which the hyphae, that have a centrifugal growth, are embedded (Fig 1). The interstitial substance is minutely lacunose, when seen under the oil immersion lens. A granular structure as mentioned by MACKINNON could not be observed in the preparations which were at our disposal, nor were any intracellular brown pigment particles to be seen. The diameter of the hyphae in the parasitic granules is rather variable. Thin hyphae, 1—2 μ in diameter, can be observed, but generally the hyphae are thicker, shortcelled, with cells 4—10 μ in diameter. Very large, isodiametric cells, attaining 18 μ, are often called chlamydospores.

Isolate number 10528, which grew from a parasitic granule, was sent to the Centraalbureau voor Schimmelcultures at Baarn, where it was identified as *Madurella species*. Because this strain was different from every strain of that species in the Baarn collection, it was subsequently sent to Professor MACKINNON at Montevideo, Uruguay, who studied it and classed it as *Madurella mycetomi* (LAVERAN) BRUMPT in his ,,Contribution to the study of the causal organisms of maduromycosis".

Cultural characters

After 22 days incubation at 25° C.

Sabouraud glucose agar. pH 5.0 (with Difco peptone) — Growth slow. Colony 12—17 mm in diameter, slightly raised, convoluted, lumpy, with tiny cracks, covered with gray to Pale Olive-Buff (XL) ¹), felty aerial mycelium. Substrate mycelium Honey Yellow (XXX), visible in cracks and between aerial hyphae near margin. Reverse Buffy Olive (XXX), margin of 1 mm Honey Yellow (XXX). Soluble pigment yellowish. Agar remaining transparent.

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¹) Numbers refer to Ridgway's Color Standards and Color Nomenclature, Wash. D.C. 1912.

Sabouraud maltose agar. pH 5.0 — Growth slow. Colony 10—13 mm in diameter, much raised, convoluted, covered with short, woolly aerial mycelium, with 0.5 mm broad ,plane margin, for the greater part deep greenish blue-gray, white towards Olive-Buff (XL) margin. With pocket-lens tiny, white tufts are visible, protruding from the margin. Reverse Citrine-Drab (XL) and Deep-Olive (XL); margin of 1 mm Olive-Buff (XL). No soluble pigment.

A 3 months old culture in a culture tube is 17—20 mm in diameter, cerebriform, felty, grayish brown, with two, moist, much raised, smooth, cerebriform, reddish brown, in the center darkbrown knobs. Soluble pigment brown. Agar remaining transparent.

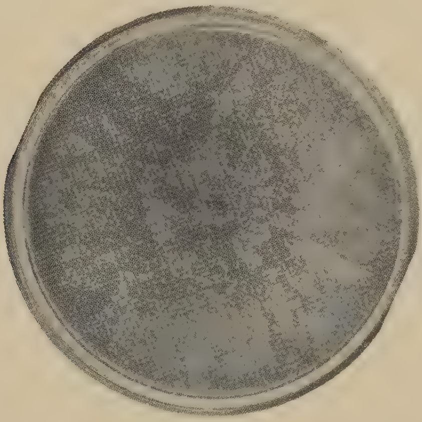

Fig. 2.

Colony of *Madurella mycetomi* (Laveran) Brumpt, strain 10528, after 20 days' incubation at 25° C. on conservation agar.

Conservation agar. pH 6.6 (Fig. 2) — Growth rapid. Colony 65—90 mm in diameter, spreading, plane, just around the inoculum smooth, Light-Buff (XV), with tiny, whitish sclerotia; rest of colony covered with arachnoid-woolly, short, white aerial mycelium, which becomes less dense towards the margin. Reverse Honey Yellow (XXX) to Olive-Ocher (XXX), lighter towards margin. No soluble pigment. Strong odor of ammonia. A 3 months old culture grown in a culture tube is felty, light gray and plane, except around the inoculum, where it is folded. The agar is light brown and slightly turbid.

Czapek agar. pH 6.6 — Growth slow. Colony 10—12 mm in diameter, 5—6 mm high, convoluted, moist, black, with tiny cracks and covered with very thin, grayish aerial mycelium; margin

Avellaneous (XL). Reverse concave, Avellaneous and black. Soluble pigment very faint, yellowish brown. No specific odor.

Wort agar. pH 7.0 — Growth slow. Colony 10—13 mm in diameter, slightly raised, covered with short, woolly, grayish-white aerial mycelium extending with small tufts beyond margin; center of colony convoluted, moist, Dusky Drab to Black (XLV to LIII); margin narrow, depressed, smooth, Dusky Drab (XLV). Reverse Mars Brown (XV) with 1 mm broad, Ochraceous-Buff (XV) margin. No soluble pigment. No specific odor.

Oatmeal agar. pH 6.1 — Growth slow. Colony 16—25 mm in diameter, plane, smooth, moist, with greenish black streaks and spots, narrow, hyaline margin and hyaline sectors.

Aerial mycelium very thin, hyaline. Reverse same color as above. No soluble pigment. No specific odor.

Cornmeal agar. pH 6.0 — Growth slow. Colony 17—20 mm in diameter, about the same in appearance as on oatmeal agar, plane, moist with mossy, Deep Slate-Green (XLVII) streaks, hyaline sectors and small black sclerotia, 0.5—1 mm in diameter. Center of colony with sparse aerial hyphae. No soluble pigment. No specific odor.

Potato-glucose agar. pH 6.6 — Growth slow. Colony 14—20 mm, raised, plane with or without folds in smooth, moist, Roman Green (XVI) margin, with pruinose, Saccardo's Slate (XLVIII) to clear green or bluish gray center. Edge entire or with small tufts extending beyond it. Reverse Dark Ivy Green (XLVI), in center raised and covered with whitish aerial hyphae. Soluble pigment very faint, greenish. No specific odor.

Littman Oxgall agar. pH 7.0 — Growth slow. Colony 12—27 mm, moist, plane, raised and convoluted in center, gray with Dark Olive-Buff (XL) spots. Tiny tufts of aerial mycelium are visible with a pocket-lens. Reverse Vinaceous Drab to Purple Drab (XLV). Yellowish zone around colony.

Potato plug + glycerine. pH 5.8 — Growth slow. Colony cerebriform, moist, Deep Olive-Buff (XL) to Citrine-Drab (XL), covered with short, woolly, white aerial hyphae, less dense towards margin. Brownish discoloration of plug just beneath colony. No specific odor.

Carrot plug. pH 5.5 — Growth slow. Colony 10—21 mm in diameter, moist, cerebriform and raised in center, Deep Olive Buff (XL), with scattered, small, black sclerotia, some hyaline aerial hyphae and a plane margin. No discoloration of plug. No specific odor.

Skimmed milk. pH 6.0 — Thick, cream colored, felty, convoluted pellicle at the surface, with small hyaline to black sclerotia in it. After 12 months, milk brown and less turbid.

Gelatin. pH 5.6, 30° C — Growth good. Colony plane, moist, light gray to light brown or nearly black, towards margin cream colored, locally with arachnoid aerial hyphae and some black sclerotia. Gelatin liquefied. Soluble pigment light brown.

Starch agar. pH 6.6, 30° C — Growth good. Colony 36—46 mm in diameter, plane, mossy, greenish black, covered with light gray, thin, woolly aerial mycelium, with black sclerotia in transparent parts of the colony. No soluble pigment. Diastatic action positive.

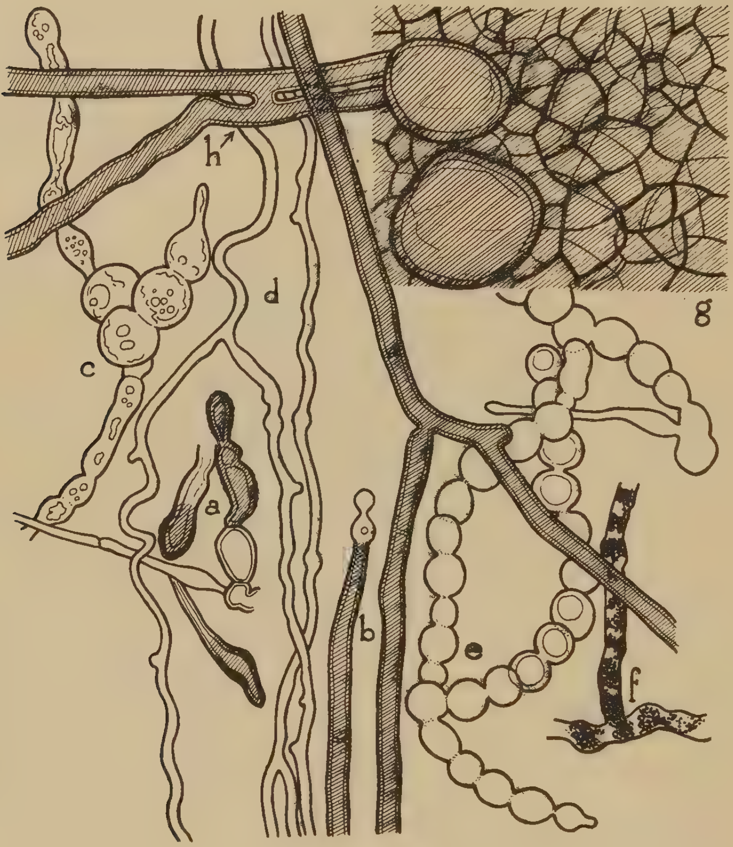

Fig. 3.

a- Hyphae with crust of pigment on the outer cellwall (from culture on potato-glucose agar).
b- Slender, dark hyphae from culture on Sabouraud glucose agar.
c- Inflated hyphae on same medium.
d- Narrow, twisted hyphae on conservation agar.
e- Moniliform hyphae resembling chains of spores (from culture on conservation agar).
f- Hypha with intracellular pigment particles (from culture on potato-glucose agar).
g- Cross section through part of a sclerotium on conservation agar.
h- Anastomosis between two neighbouring hyphae.

Microscopical characters

On Sabouraud glucose agar two types of hyphae are to be seen, one type showing long, dark brown hyphae of equal thickness (3.5—4.5 μ), usually with septa at more or less long intervals, with only few branches (Fig. 3-b), the other type showing irregularly inflated, hyaline hyphae, up to 9 μ in diameter (Fig. 3-c). Anastomoses were observed between the slender, dark hyphae (Fig. 3-h). The microscopical characters on conservation agar are almost the same as on Sabouraud glucose agar, except as for the abundant production of globular, eggshaped or irregular, black sclerotia 200—400 μ in diameter, which are pseudoparenchymatous and which often have bigger, globular cells in the interior (Fig.3-g) and the presence of a delicate mycelium consisting of narrow, twisted, hyaline, profusely branched aerial hyphae, 1—2 μ in diameter (Fig, 3-d). The shortcelled hyphae are composed of almost globular cells and resemble chains of conidia (Fig. 3-e). These short cells are often called oidia. On potato-glucose agar hyphae are observed either with a fairly thick extracellular crust of brown pigment or with intracellular pigment particles (Fig. 3-a and -f).

The above-described strain is for short characterized by the aspect of its cultures on potato-glucose agar, conservation agar, oatmeal agar and wort agar, being blueish and crustlike on the first, spreading, arachnoid and cream-colored on the second, black and moist on the third and grayish brown, felty on the last medium. The very faint pigment production into the medium and the rapid development on conservation agar also typify it.

Summary

A report is given of a case of black grain maduromycosis in a 38 year old man living on Curaçao, Netherlands Antilles. The silver-impregnation method for staining reticulin is successfully used for staining of the fungi in tissue sections. The causative fungus is *Madurella mycetomi* (Laveran) Brumpt. The isolated strain differs from the usual isolations of this species by its very faint pigment production into the medium. The cultural and microscopical characters of this strain are described.

Literature

Mackinnon, J. E. (1954), A contribution to the study of the causal organisms of maduromycosis, Trans. Royal Soc. Trop. Med. Hyg., 48,6 470—480.

PRECIPITIN STUDIES FOR THE IDENTITY OF ANTIGENS IN DIFFERENT SPECIES OF SPOROTRICHUM [1])

BY

RUDOLF H. KADEN, M. D. [2]).

(8.VIII.1957)

(with 4 figs.)

I. Introduction

CONANT (1954) considers only one species, *S. Schenckii*, as the etiologic agent of sporotrichosis. This conception is based on several observations made during the investigation of *Sporotrichum* strains. In agglutination tests LURIE (1948) demonstrated a common antigenic factor in different species of *Sporotrichum*. NORDEN (1951) extended the serological studies by using precipitin tests. He succeeded in showing precipitation between antigens of *Sporotrichum* species and their antibodies. Improved methods of demonstration antigen-antibody reactions has been shown by the use of agar diffusion methods (OUCHTERLONY (1949), SEELIGER (1954), WILSON & PRINGLE (1954), and KADEN (1956)). This precipitin test in agar medium was used in the studies for the identity of antigens in different species of *Sporotrichum*.

II. Materials and Methods

The following five *Sporotrichum* strains were selected for the examination [3]):

No	classification	origin
1	*S. Schenckii*	human case, Duke Hospital, N.C.
2	*S. Beurmanni*	collection of WESTERDIJK, Baarn, Holland
3	*S. asteroides*	collection of WESTERDIJK, Baarn, Holland
4	*S. Schenckii*	human case, N.C. Baptist Hospital, N.C.
5	*S. Schenckii*	human case, Boston, Mass.

The colonies of these strains on Sabourauds agar maintained at room temperature showed a great variation in pigmentation and their colors differed from dark brown to white.

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[1]) This work was done during a Fulbright Fellowship spent with Dr. NORMAN F. CONANT, Department of Bacteriology, Duke University School of Medicine, Durham, N.C.

[2]) From Hautklinik der Freien Universitaet Berlin im Rudolf Virchow-Krankenhaus, Germany.

[3]) Obtained from the laboratory of Medical Mycology maintained by Dr. NORMAN F. CONANT, Duke University.

a) *Antigens*

1. Dissolved autoclaved antigen

Based on the report of SEELIGER (1955) dissolved autoclaved antigens were used for the precipitation. The *Sporotrichum* strains were cultured on Brain Heart Infusion-blood agar at 37° C to obtain the yeast phase. A cell suspension in saline at a concentration of 1 : 20 was prepared and autoclaved for 15 minutes at 120° C and under 15 lbs pressure. The residue of organisms was eliminated by centrifugation [1]) for 30 minutes. To the supernatant fluid was added 4 volumes of 95% alcohol to precipitate the antigen in it. After leaving over night in a refrigerator the antigen had become a white, fluffy sediment at the bottom of the flask. The supernatant fluid was decanted and only a sufficient amount of saline was added to redissolve the sediment. The solution was practically clear and was the dissolved autoclaved antigen.

2. Dissolved sonic vibrated antigen

Living yeast phase organisms of the same strains were suspended in sufficient amount of saline to give a packed cell volume of 1 : 20. A sample of 20 ml was transferred to the treatment vessel of a sonic vibrator [2]). After a treatment of 30 minutes the suspension appeared to be finer grained; however, no change could be observed by microscopical examination. The sonic vibrated antigen was stored for 6 weeks at —20° C. After thawing the suspension, it was centrifuged for 30 minutes at 3000 r.p.m. The supernatant fluid was treated as described above, with 95 % alcohol, separation of the sedimentated antigen, and redissolution with saline. The amount of precipitate formed by the addition of alcohol was remarkable great. The solution obtained was practically clear and was the dissolved sonic vibrated antigen.

b) *Antisera*

1. Immunisation of rabbits

Healthy rabbits, weighing about 4500 g, were immunised for the production of *Sporotrichum* antiserum. Agglutinin titrations were negative in each animal before the injections were started. The rabbits and the antisera were labeled so as to correspond with the antigens:

Culture	Antigen	Rabbit	Antiserum
S. Schenckii	No 1	I	I
S. Beurmanni	No 2	II	II
S. asteroides	No 3	III	III

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[1]) In an International, size II, centrifuge at 3000 r.p.m. at room temparature.

[2]) „Raytheon" oscillator, type R—22—3, oscillation rate 9000 cycles per second.

According to the experiences of NORDEN (1951) a suspension of the yeast phase organisms was raised to a concentration of ten Times McFarland scale No 5. After killing the organisms by the addition of 0.3 ml formalin to 100 ml of each suspension, a vaccine for immunisation was manufactured and referred to as formalinised antigen. Sterility tests were made of the formalinised suspensions to assure complete lack of viability. In order to secure high titers in the immunised animals, an intensive injection schedule was adopted after studing the results reported by LURIE (1948), NORDEN (1951), and RÉ (1955). Equal amounts of the vaccine were injected intravenously and intraperitoneally at the same time. The following volumes were given: 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 3.2 ml. After an interval of 14 days the second series of injections were started. 1.0, 2.0, 3.0, 3.0 ml were given similarily. The rabbits were bled one week after the last injection. One rabbit, however, required a third series of injections of 3.0, 3.0, 3.0 ml to obtain a suitable agglutinin titer in its serum. The rabbits immunised according to this injection schedule did not show any signs of overdosage. They remained healthy and they even gained weight.

2. Agglutinin titer in the antisera

For the determination of the agglutinin titer in the antisera of the immunised rabbits agglutination tests were carried out according the techniques reported by CONANT (1954). These tests require an autoclaved *Sporotrichum* suspension for the use as antigen.

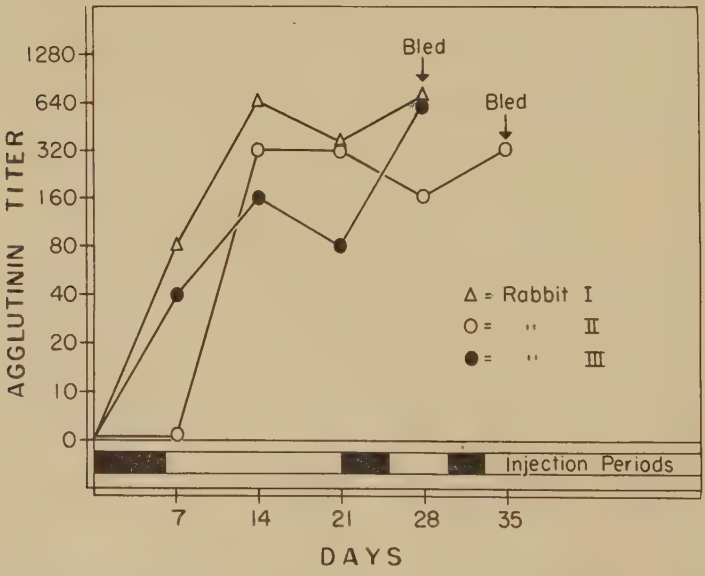

Fig. 1. Agglutinin titer in rabbits to homologous *Sporotrichum* antigens during the injection period.

After its dilution to 1 : 1000 it was referred to as heatkilled *Sporotrichum* vaccine. The titer in the rabbit sera was determined every seven days. The production of antibodies was shown to depend upon the individual response of the immunised organism against the injected antigen (Fig. 1). 14 days after the start with injections, rabbit I and rabbit II responded with their highest titers which they never surpassed. After the second injection series, in 28 days, the titer in rabbit I and rabbit III has risen to 1 : 640. In this moment the rabbits were bled to obtain a *S. Schenckii* antiserum of 1 : 640 and a *S. asteroides* antiserum of 1: 640. The titer in rabbit II did not regain the dilution 1 : 320 before a third injection series, in 35 days, was added. After bleeding rabbit II a *S. Beurmanni* antiserum of 1 : 320 was obtained for the precipitin studies.

c) *Precipitin tests*

1. In agar medium

20 ml agar medium [1]) was allowed to solidify in a Petri dish. With the aid of a cork borer No 8 one hole was made in the centre and four or six holes were made at 5 mm distance around the centre hole. 0.2 ml of the testing material (antigen or antiserum) was placed in each hole which now acts like a reservoir. The material was used undiluted. The reservoirs were numbered clockwise. After a two day period precipitin lines in the agar medium were beginning to form. Readings were made after a 7 day incubation period at room temperature and/or 5 days at 4° C. Refillings were avoided to guarantee exact results. This technique had been worked out on the basis of reports about details and variations of the precipitin test in agar medium by Wilson & Pringle (1954), Seeliger (1956), and Kaden (1956).

2. In capillary tubes

For precipitin test in capillary tubes special tubes of about 6 cm in length and 3 mm in diameter were used. Every tube was filled with 0.1 ml *Sporotrichum* antiserum using a tuberculin syringe. The same amount of *Sporotrichum* antigen was accumulated likewise upon its surface. The tubes were allowed to stand for ½ hour in room temperature. After this period the results were read. Final readings were made after 2 and 24 hours respectively and storage in a refrigerator at 4° C. At the contact area of the two reagins an opaque disciforme zone of different intensity demonstrated a positive precipitin result.

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[1])

Bacto-agar „Difco" standardized	20.0
NaCl	8.0
Merthiolate sol. 1 : 1000	100.0
Bromphenolblue sol. 1 : 100	0.1
Destilled water	ad 1000.0

d) Precipitin absorption tests

For specifity precipitin absorption tests were carried out. Capillary tubes as descriped above, and Waterman tubes were used for the demonstration of precipitation. At the beginning of the absorption test Waterman tubes were filled with 0,5 ml *Sporotrichum* antiserum and with equal amounts of *Sporotrichum* antigen. The material was allowed to precipitate for 24 hours. The precipitated sediment was then removed by centrifugation for 10 minutes at 3000 r.p.m.. The clear overlaying fluid represents the absorpted *Sporotrichum* antigen.

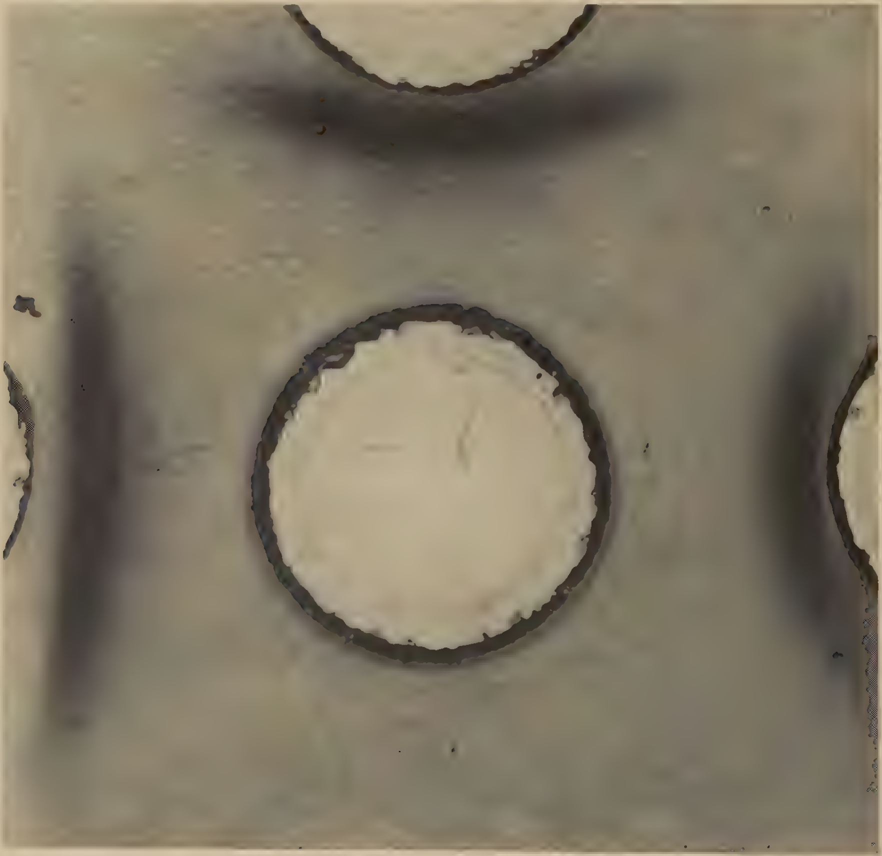

Fig. 2. Similar precipitin lines between *Sporotrichum* antiserum and antigens of *S. Schenckii*, *S. Beurmanni*, and *S. asteroides*.

centre: *Sporotrichum* antiserum
left: Antigen of *S. Schenckii*
above: Antigen of *S. Beurmanni*
right: Antigen of *S. asteroides*

In the second step of the procedure capillary tubes were used to be filled with *Sporotrichum* antiserum and accumulated with absorped *Sporotrichum* antigens. The precipitin test was observed 30 and 120 minutes. The final reading was made after 24 hours according to the instructions about the precipitin test in capillary tubes.

III. Results

In agar medium none of the dissolved autoclaved antigens, No 1 through No 5, showed any significant difference in its precipitin lines when tested against each of the *Sporotrichum* antisera, I, II, III (Fig. 2). The lines converge to their middle portion into a more or less intensive band. Towards the periphery the lines separate and form shapes suitable for the identification of the antigen-antibody system being involved. Three lines of the precipitin band are significant in their characteristic shape and intensity. Two of them run nearly parallel and are situated close to the antigen reservoir. Their intense formation gives rise to form arcades by fusing with the adjoining corresponding lines. The third characteristic line is isolated from the precipitin band and is situated close to the antiserum reservoir. Its shape forms a practically straight line, and its intensity is too low as to form arcades with the adjoining corresponding line. This line has scarcely developped in antigens of *S. asteroides*. Occasionally the precipitin formation may differ in its intensity; however, in the examination of *Sporotrichum* species these three characteristic lines used to be found.

A significant difference in the intensity can be provoked by carrying out the precipitin test at different temperatures. At 4° C it takes a considerable shorter period to obtain visible precipitin lines, and above all their intensity becomes higher than at room temperatures. A reading after 5 days at 4° C indicates the characteristic shape of the precipitin formation much clearer than at room temperatures. Even the three precipitin lines for *Sporotrichum* will usually be observed when a magnifying glass is used for the readings. (Fig. 3). In contrary the precipitin lines at room temperature after 5 days are still feeble and do not allow to observe further differentiation. In addition to that advantage a smaller amount of reagin liquid is lost by evaporation at 4° C incubation temperatures. The reservoirs used to maintain a rest of reagin liquids for 5 to 10 days. Therefore all reservoirs contain still sufficient reagins at the reading time in 5 days.

Sonic vibrated antigens in comparison to autoclaved antigens show a somewhat changed and more intensive precipitin formation (Fig. 4). Beside an elongation of the original precipitin lines a second precipitin band runs parallel at the side towards the center hole where the antiserum is placed. This band does not form arcades

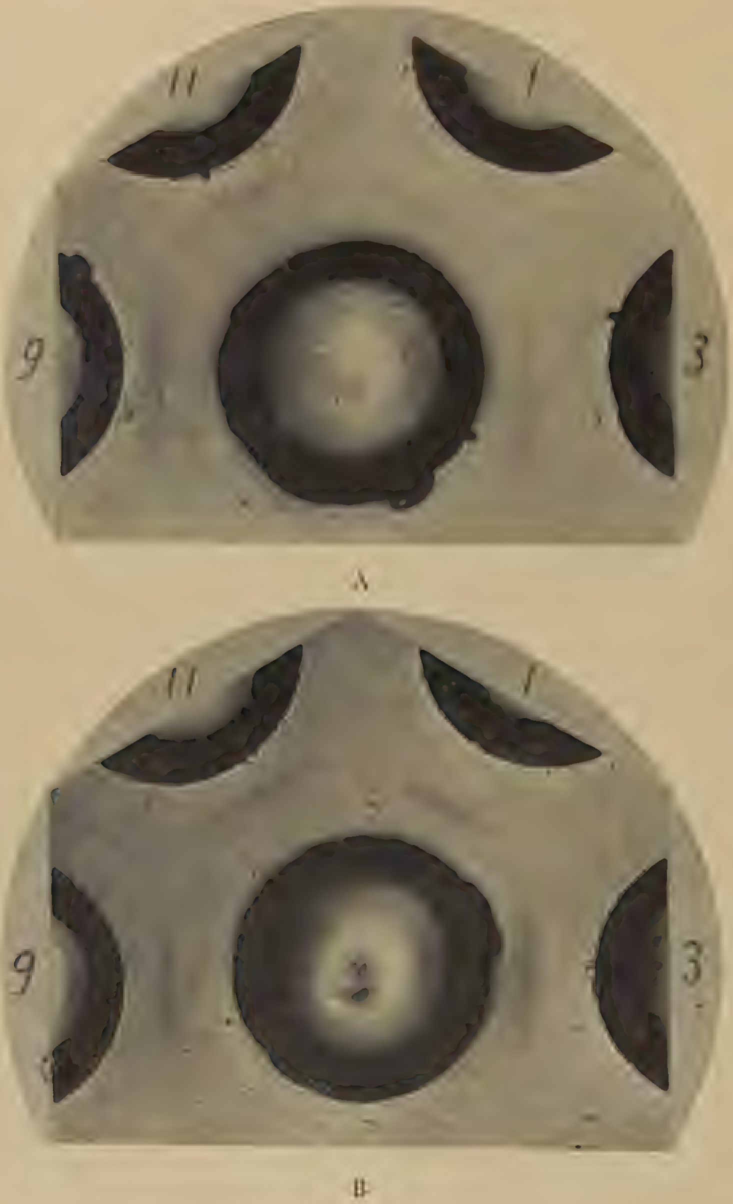

Fig. 3. Increased intensity of precipitation by incubation temperature of 4° C.
A. Precipitation in room temperature.
B. Precipitation in 4° C.
Reservoir # 1: Antigen #4 of *S. Schenckii.*
Reservoir # 3: Antigen #5 of *S. Schenckii.*
Reservoir # 9: Antigen #1 of *S. Schenckii.*
Reservoir #11: Antigen #1 of *S. Schenckii.*

with the neighboring precipitin band caused by autoclaved antigens. The precipitin lines between sonic vibrated antigens of different *Sporotrichum* species are as similar as the precipitin lines between autoclaved antigens. There is an obvious difference in the precipitin formation resulting from antigens of the same strain but produced by different technical methods.

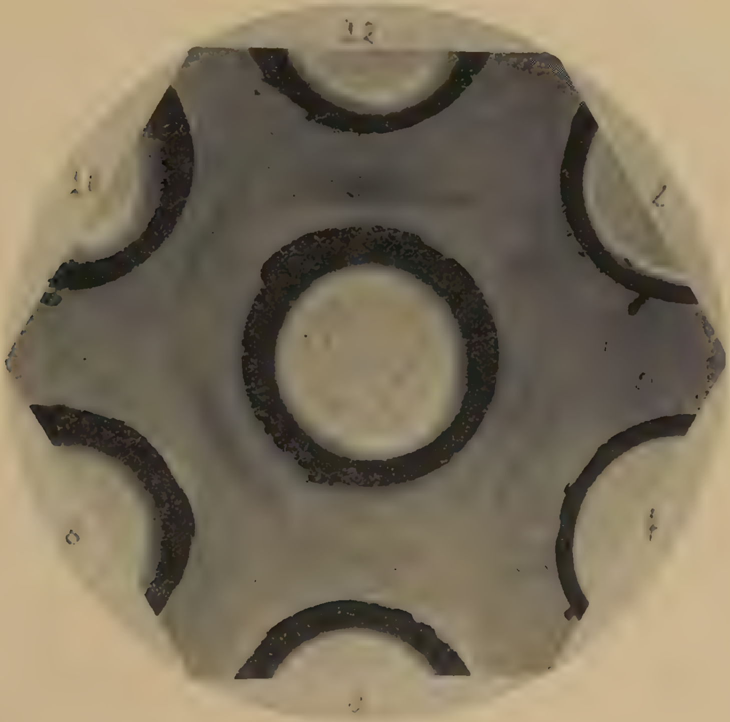

Fig. 4. Difference in formation of precipitin lines with autoclaved and sonic vibrated *Sporotrichum* antigens.
Reservoir # 2: Antigen of *S. Schenckii*, autoclaved.
Reservoir # 4: Antigen of *S. Schenckii*, sonic vibrated.
Reservoir # 6: Antigen of *S. Beurmanni*, autoclaved.
Reservoir # 8: Antigen of *S. Beurmanni*, sonic vibrated.
Reservoir #10: Antigen of *S. asteroides*, autoclaved.
Reservoir #12: Antigen of *S. asteroides*, sonic vibrated.

The results of precipitin reations in capillary tubes of the three different species of *Sporotrichum* with each of the *Sporotrichum* antisera correspond fundamentally with the results of the precipitin test in agar medium. The capillary precipitin test has prooved to give distinct precipitin reactions even if antisera of extreme low titer or diluted antigens are used; however, the capillary method does not give differentiated details of the reacting antigen-antibody system.

Absorption experiments in the precipitin test with capillary tubes are not satisfactory. Even an autoclaved antigen already absorbed by its homologous antiserum would again precipitate with the very same antiserum.

IV. Discussion

The shape of precipitin formation in agar medium is said to depend on the reacting factors of the antigen-antibody system if dissolved antigens and high titer antisera are used (RAFFEL (1953).) The specifity of the precipitin effect has been discussed in the experimental experiences of WILSON & PRINGLE (1954) and SEELIGER (1955), and is demonstrated by our results in controls with low or no titer antisera. In accordance with previous reports there is no doubt that precipitation in agar medium requires higher titers in antisera and a saturated concentration in antigens than precipitation in test tubes. RAFFEL (1953) indicates a ten times higher sensitivity of the precipitation results if capillary tubes are used. On the other hand the advantage of the precipitation in agar medium can't be neglected. The formation of differentiated precipitin lines gives certainly much better information about the antigenic structure of a fungus than the qualitative results in capillary tubes; however, the interpretation of the precipitin lines formation requires some consideration. The demonstration of temporary variability in the precipitin lines caused by temperature, concentration of reagines, techniques used in their production has to be considered carefully. Observing these facts exact informations about the antigenic structures will be obtainable from the shape of the precipitin lines. In this connection the similarity in the precipitin formation of *S. Schenckii*, *S. Beurmanni*, and *S. asteroides* interpretes their antigenic identity. These findings agree with those of LURIE (1948) and NORDEN (1951) who, in agglutination tests, came to the conclusion that there is at least one common antigenic factor in different species of *Sporotrichum*.

V. Conclusion

The antigenic structures of different species of *Sporotrichum* can be demonstrated and studied using the precipitin test in agar medium. Insignificant differences in precipitin lines formation are shown to depend upon temporary factors. They do not involve the interpretation of the precipitin results concerning their identity of their findings. Dissolved sonic vibrated antigens cause other precipitin lines formation than dissolved autoclaved antigens, even if both antigens are originated from the same strain. The similarity of the precipitin lines in the three different species of *Sporotrichum* and their interpretation agree with the antigenic identity of *S. Schenckii*, *S. Beurmanni*, and *S. asteroides*.

Summary

1) A simple technique for the preparation of dissolved autoclaved antigens and of sonic vibrated antigens, the immunisation of rabbits, and testing methods by precipitation in agar medium and in capillary tubes are described.

2) Five different strains of *Sporotrichum* belonging to three species, *S. Schenckii*, *S. Beurmanni*, and *S. asteroides* are studied as to their precipitin lines formation in agar medium.

3) The similarity in precipitin lines formation is interpreted to demonstrate the antigenic identity of these different *Sporotrichum* species.

4) Comparable results with minor differentiation but in major sensitivity are obtained by precipitin tests in capillary tubes.

5) Temperatures of 4° C are shown to stimulate intensity of precipitin lines.

6) Dissolved sonic vibrated antigens alter the precipitin formation by changing and intensifying the precipitin lines.

7) Absorption experiments with dissolved antigens give unsatisfactory results if precipitin tests in capillary tubes are used.

Acknowledgments

The author wishes to acknowledge gratefully the facilities and hospitality given to him by Duke University School of Medicine. He further is indebted to Dr. NORMAN F. CONANT for his invaluable advise and sustained interest; to Dr. HILDA POPE WILLETT for kind help in the precedure of sonic vibrated antigens; to C. M. BISHOP and P. FAIRCHILD, both from the Department of Medical Illustration for the photographies and drawing respectively.

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A CRITICAL REVIEW OF MEDICAL MYCOLOGY IN THE UNITED STATES 1946—1956.

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(16.X.1957)

MONOGRAPHS AND TEXTBOOKS

Medical mycology in the U.S. has kept pace — to say the least — with the tremendous progress accomplished in this country in other branches of medicine. Several monographs and textbooks have appeared (10, 16, 19, 20) of which CONANT'S manual (3) has become "the" standard text in all laboratories. This small book contains a huge amount of factual information, which is presented simplified and clearly; this is a feature also in several contributions by CONANT to textbooks of bacteriology (4,22). LEWIS & HOPPER have produced several editions of a textbook on medical mycology directed especially to the dermatologist (7). The former and the atlasses of MOSS & MCQUOWN (8) and the one of HAZEN & REED (5) are beautifully illustrated books. The importance attributed to fungus diseases finds expression in a small monograph directed to the general practitioner (21) in rather extensive chapters on mycology in textbooks of dermatology (11, 18), pathology (1), and bacteriology (4, 22), and to lesser degree in internal medicine (2), pediatrics (9) etc. Proceedings of three major symposia cover mycology in general (13), histoplasmosis (12) and therapy of fungus diseases (17). The textbook edited by SIMONS (15) with contributions of 36 international authors has chapters which vary in quality. The recent book of SCHMIDT (14) has only a few good contributions. A "practical medical mycology" was written by KEENEY (6).

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NORTH AMERICAN BLASTOMYCOSIS

Several review articles are available (7, 8, 22, 35, 57, 59, 69, 76). Serologic studies (6, 17, 20, 21, 31, 52) revealed certain cross reactions between sera of animals sick with *Blastomyces dermatitidis*, *B. brasiliensis* and *Histoplasma capsulatum*. Fractions were isolated from blastomycin (14, 15, 40) and their behavior studied. In vitro testing of growth requirements (24, 25, 27, 50) and of anti-fungal agents showed the most impressive inhibition (prior to the antibiotic amphotericin B) with diamidine derivatives (12, 13, 68).

Unusually small forms of the organism in tissue have been repeatedly reported (39, 57, 73, 74) which is especially important in reference to dual infection (36) or confusion with other yeast organisms. Combinations with tuberculosis (4, 70) and sarcoid (29) have been seen.

The greatest single event in this decade was the introduction by Schoenbach (53, 54, 55) of stilbamidine for the treatment of the disease. For the first time disseminated cases became arrested due (7, 10, 11, 18, 28, 41, 45, 47, 48, 64, 65, 67, 72, 77), and not in spite of treatment (3, 30, 32, 42), even if recurrences (28, 34) and neurotoxic damage (19, 28, 65) were reported. A few lethal cases have been ascribed to toxicity from stilbamidine (10) which in view of the easy decomposition of the unprotected drug is well understandable. Antifungal antibiotics of low toxicity will present the definitive answer to the problem of treatment. A new concept of pathogenesis was introduced (57, 59) pointing to the lung as portal of entrance in almost all cases, leaving the cutaneous manifestations as metastatic. The only documented primary inocculations have so far been observed in the laboratory (57, 59, 60, 80). Many have accepted the fact of the pulmonary origin, but the discussion has not died out yet (38).

This concept brings blastomycosis in line with histoplasmosis and coccidioidomycosis (61); organisms have been found in the two latter diseases in the soil and the epidemiologic (66) anatomical (51, 57) and clinical experiences (2) point clearly to the respiratory tract as regular portal of entrance. The existence of subclinical cases of blastomycosis similar to the ones observed in coccidioidomycosis (61) and histoplasmosis (9, 37, 71) seemed unlikely from a major survey with skin testing antigens in the Cincinnati area (23) but certain data have been recently presented (66) which if confirmed on a larger scale and with standardized antigen would indicate that at least in a highly endemic area subclinical forms of blastomycosis may exist.

The relative frequency of the disease in the USA was documented with the results of an epidemiologic inquiry (62). The first and only verified "epidemic" was observed in a small village in North Carolina (66) but all efforts to isolate the organism, from soil or water or to establish human or lower animal sources for transmission failed. The disease is not uncommon in dogs in the endemic area (44, 46, 49, 51) but no transmission has been documented and one case supposedly originating after a dog bite (63) is certainly not at the level of Cesar's wife. An "epidemic" reported from Okinawa (5) lacks objective proof (56).

Inspite of many efforts the organism has not been isolated from nature, but its growth under experimental conditions in sterilized soil and on tree bark has been accomplished (16, 43).

In view of the increasing activity of chest surgeons considerable experience has accummulated (1, 33, 75, 78, 79) about the benefits of surgical removal of focal pulmonary lesions (which clinically and radiologically often were considered to be carcinoma preoperatively). When combined with diamidine treatment (1, 35, 41, 79) results have been on the whole encouraging. The strictly pulmonary character often seen in such cases — in absence of any cutaneous manifestations — is one of the points for the above mentioned opinion (57, 59) of the pulmonary primary infection in blastomycosis.

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CANDIDIASIS

The widespread use of antibiotics with the supression of bacterial organisms has contributed to more frequent recognition of the presence of *Candida* in sputum and other materials, which has been accepted by many workers as proof of an increase in the number of candidiasis (27). Experimentally attempts have been made to show a relationship between antibiotics and candidiasis (2, 9, 20, 33, 37, 43). Most medical mycologists are very skeptical about the importance of the presence of *Candida* in clinical material and the problem is one of interpretation rather than of recognition of *Candida*. (23). Therefore many cases diagnosed as broncho-pulmonary candidiasis have no more proof than the presence of *Candida* in sputum or bronchial secretions (3, 21, 31, 45). It seems that only the demonstration of *Candida* in lung tissue removed by open biopsy can be accepted as scientific proof of its pathogenetic importance (23).

While *Candida albicans* is usually considered the only pathogen of the species, lately reports have appeared of disease processes from which *C. krusei* (44), *C. guilliermondi* (46) and *C. parapsilosis* (5) were isolated.

The greatest single event of the decade was the isolation of nystatin by Hazen & Brown from *Streptomyces noursei* (17). This potent antibiotic has given excellent results experimentally in vitro (18) in vivo (2) and most important in clinical medicine (6, 14, 32).

Other materials have been used for treatment experimentally (24) and clinically (19).

The production of chlamydospores, a crucial point in the identification of *C. albicans* has been accomplished by Reid et al (35) using zein containing medium instead of whole corn meal agar and by Nickerson (29) on completely synthetic media. The relative value of various types of corn meal media has been investigated (13). The effect of cysteine on *C. albicans* was noted (41). Folic acid analogues inhibited the growth of *C. tropicalis*, the effect being reversed by the addition of folic acid to the media (29, 30). The pathology of experimental candidiasis was clarified (1, 10).

A comprehensive review of the *Candida* group of organisms including biologic reactions, taxonomy and clinical effects was made by SKINNER. (38).

Of interest clinically have been reports of nasolacrimal duct obstruction (8), corneal infection (28) and oral and pharyngeal lesions (45).

Many disseminated cases have been reported (11, 36) especially in drug addicts (26, 46). Endocarditis (12, 22, 26, 44, 46) and meningitis (12, 16, 34, 39, 47) have been prominent findings. Association with blood dyscrasias has been noted (7, 25). Combined infection with *Cryptococcus neoformans* has been reported (39, 40). Other debilitating diseases have been associated (11, 40). A post cesarean section generalized infection has been observed (4). *Candida* has been cultured from a myxoma of the auricle post mortem in one case (5).

In a routine study of autopsies for *Candida* positive cultures were obtained in several instances from lungs and once from liver, spleen etc. (15).

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COCCIDIOIDOMYCOSIS

Several reviews of coccidioidomycosis have appeared (3, 6, 12, 30, 76, 78, 79, 103).

Coccidioides immitis was found in 7% of 500 soil samples in the San Joaquin Valley, mainly in burrows (25). Spherules of the organism have been grown in vitro by several workers (9, 17, 56, 66, 97). Conversion of hyphae to spherules in vitro and in vivo appeared to result from inhibition of hyphal growth followed by development of spherules on the stunted hyphae (2). Detailed morphologic studies of 47 strains of *C. immitis* have been reported (32). Interesting findings were mycelial forms in surgical specimens and sputa (29, 30, 39, 74). Culture of the organism from air is best accomplished using actidione in the media (35). One positive blood culture has been reported (28). Study of both spherule and hyphal forms of *C. immitis* by electron microscopy was carried out by O'Hern & Henry (68). Dried spores of *C. immitis* proved adaptable to temperatures ranging from -15°C to 37°C with humidity varying from 10-95%. 50°C proved lethal but only after one week. Suspension in saline at low temperatures impaired survival (34). Burke was able to culture a sealed spherule mount seven months after the mount was made (9). Atypical growth of *C. immitis* was observed by Spaur (89). The use of embryonated eggs as a culture medium for *C. immitis* has been described (8, 36, 66, 97). Apparent budding of spherules was described by Creitz in mounts of material from human and experimentally infected animals (22). The use of 2-4 day old cultures of *C. immitis* for animal innoculation was considered safe and effective diagnostically (23). Hampson noted diminished spore formation of 20 strains when incubated in screw top as compared with cotton plug bottles and at 37°C in contrast to room temperature (40). Spherules of *Coccidioides* contain chitin and are surrounded by a phospholipid while those of *Haplosporangium parvum* contain cellulose and have no phospholipid sheath (7).

Histologic details of experimental infection in mice have been reported (30, 94). The experimental production of granuloma and subcutaneous air pouches in rats was reported by WRIGHT (106). No relation between virulence and facility of conversion from spherules to mycelial phase was noted (70). Some protection was afforded both mice (33) and guinea pigs (98) by injection of killed vaccine prior to challenge with virulent *C. immitis*.

Details of interpretation of the coccidioidin skin test have been worked out (84, 87, 101). Use of triple antigen in 12,099 instances has been noted (64). The complement fixation test is very valuable in diagnosis and prognosis while the precipitin test is mainly diagnostic in significance (75, 85, 101).

The localization of endemic areas has revealed several outside of the original area in the San Joaquin Valley (5, 41, 55, 80). The exceptional appearance of disease in persons outside the endemic areas has been reported (4, 12, 61, 77).

The enzootic areas correspond to endemic areas and infection is seen in many animals especially in dogs and cattle (18, 46, 47, 53, 57, 58, 73, 87, 90, 93, 99).

Numerous clinical and epidemiological studies have been made by SMITH and his group (79, 80, 81, 82, 83, 84, 86). The frequent occurrence of erythema nodosum with primary disease and its recurrence after sand or dust storms has been emphasized by COHEN (13). The pattern of the disease in children has been well defined (27, 62).

Disseminated disease with and without specific localization is discussed by many authors. (4, 10, 12, 28, 31, 38, 43, 50, 52, 59, 60, 67, 69, 71, 95, 105). The relation of coccidioidal infection to pregnany was mentioned by VAUGHAN and complement fixing antibodies have been demonstrated in the newborn (13, 15, 96).

Coexistence of coccidioidomycosis with tuberculosis (21, 31, 44, 91) and with sarcoid (26) has been observed. An exceptional case of primary skin disease has been reported (102).

Emphasis has been placed on the pulmonary residuals of primary infection with *Coccidioides immitis* (19, 42). Complications of pulmonary cavitation per se were outlined by COTTON et al. (19, 20). Cavitation generally is considered benign (72, 81, 104). Whether reactivation occurs (45, 49), whether collapse therapy is of value (104) and whether hemoptysis is a positive indication for resection are the controversial points of the surgical discussion (1, 19, 30a, 100, 104).

The most extensive work on pathology was done by FORBUS & BESTEBREURTJE (30). WILSON et al insisted on the primary pulmonary nature of the disease (102) which was documented anatomically in men (92) and animals (93).

Treatment of disseminated disease has been generally unsuccessful (10, 16, 24, 31, 43, 48, 51, 63, 71, 88, 95). Cortisone seemed to alter the course of erythema nodosum favorably without disseminating the infection (54). Experimentally nystatin(37), diethylstilbestrol (14), stilbamidine, thorazine, pyrrolidino proprionyl phenathiazine and pentochlorophenyl cellosolve (11) and sulfonamides plus testosterone (51) proved relatively effective. Cortisone had little effect on experimental disease (65).

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CRYPTOCOCCOSIS

Old and new developments are summarized and interpreted in the excellent monograph of Littman & Zimmerman "Cryptococcosis" (32). An epidemiologic milestone was the isolation of *Cryptococcus neoformans* from soil by Emmons (16, 17) who subsequently obtained the organism in droppings from pigeons (19). The presence of pulmonary lesions with or without cerebral involvement points towards the lung as the portal of entry (2, 6, 22, 25, 36, 41, 44) for the fungus, analagous to blastomycosis, histoplasmosis and coccidioidomycosis (41).

Cryptococcosis was reported spontaneously occurring in dogs (42), cats (26), monkeys (43) but the main event to be recorded was the occurrence of epidemics of mastitis in cattle (7, 18, 35), interestingly without infection of the farmhands. The change of the milk to a viscid fluid is illustrated in Pounden's (35) paper.

The human cases in general showed the well-known central nervous system involvement (8, 9, 11, 22, 31, 36, 45, 47) but less familiar localizations, like the adrenals (37), skin, mucosae (10) and bone (12) have been reported.

Laboratory diagnosis and cultural characteristics have been advanced especially by Benham (4, 5, 38, 46) and others, who demonstrated that *Cryptococcus neoformans* does not utilize KNO_3 (if offered as the sole source of nitrogen) or lactose (serving as the sole carbohydrate source). Galactose is utilized in similar conditions. These metabolic characteristics plus growth of the organism at 37°C can be considered diagnostic for the pathogenic variety of *Cryptococcus*. Carbohydrate fermentation is not reliable and therefore not useful for diagnostic work.

The immunology of cryptococcosis and of fractions of the polysaccharides obtained from the capsule of the organism were studied. (15, 20, 21, 28, 34). An endotoxin was presumably demonstrated by Salvin (40).

Considerable attention was paid to the coexistence of cryptococcosis and malignant lymphoma, especially Hodgkin's disease (13, 23, 39, 48) and hemolytic anemia (1). While the fact of coexistence is undeniable nothing is known about the true relationship between the two diseases. In sarcoidosis (a diagnosis which is very difficult to make and to prove) some verified instances of cryptococcosis (13) are on record, sometimes following corticosteroid therapy. Combination with tuberculosis was also reported (14, 30). "The" staining technique for the demonstration of cryptococci in tissues is the muci-carmin procedure (27, 32).

The treatment of the disease has been generally unsatisfactory (3, 9, 24, 29, 33) and actidione (8, 47) has not been proven superior to previous agents used for treatment as evidenced by 11 deaths and only two remissions in 15 cases summarized by LITTMAN & ZIMMERMAN (32). Amphotericin B which is being tried at present seems to offer real hope.

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HISTOPLASMOSIS

The amount of work and progress accomplished in the last ten years in the field of histoplasmosis is one of the major milestones in modern medicine in general (112). Histoplasmosis was considered to be a rare and essentially tropical disorder but this changed radically when CHRISTIE (15) showed that subclinical forms exist which was confirmed by many authors (4, 13, 14, 38, 43, 44, 46, 47, 48, 81, 82, 91).

It became obvious that a disease which attacks such large groups of people must produce also clinical pictures hitherto attributed to other entities, especially tuberculosis. The distribution of the disease in the United States was studied and an endemic area established which includes roughly the Mississippi Valley where skin test reactivity may be found in more than 80% of the population (26, 46, 53, 71, 74, 77, 81, 82, 95, 102, 131, 136). The organism was demonstrated by EMMONS (29) in soil for the first time from a "mound of earth at the entrance of a rat burrow under the edge of a chicken house" (112). Numerous subsequent attempts to culture *Histoplasma* from soil, from silos, chicken houses, caves have been positive and much higher incidence was generally obtained from soil in the vicinity of chicken houses than from any other point source. (2, 3, 68, 72, 73, 132, 133).

Once the presence of the organism in soil was established, it became fairly easy to understand why local outbreaks occur. The epidemics were in great part elucidated by FURCOLOW, GRAYSTON, LOOSLI and others (1, 9, 10, 12, 33, 35, 41, 45, 52, 65, 73, 83, 128) who could demonstrate the connection between even short stay in storm cellars, silos, hollow trees, cleaning of chicken houses etc. The general pattern points toward inhalation of spore containing dust in such places. Demonstration of *Histoplasma capsulatum* in air samples in the endemic area (61, 97) and specifically in places where focal outbreaks of the disease originated (61) proves conclusively, from the epidemiologist point of view, that the airways are the portal of entrance which indeed is confirmed by radiologic and anatomical experiences in men (13, 33, 35, 42, 44, 72, 109, 121, 122) and animals. (30, 31, 113). CHRISTIE (13) and SCHULZ (109) had observed occasionally primary foci in the lungs of children but it was the work of STRAUB (122) who demonstrated the presence of histoplasmic primary complexes in lungs at autopsy proving: (a) that the portal of entry of the infection is the lung and (b) that in the endemic area almost 70% of unselected autopsies have residual lesions of histoplasmosis. The development of modern staining techniques was greatly contributory to this work (55, 56, 63, 85). At the time of the primary infection apparently invasion of the blood stream is common as the findings of calcified splenic lesions in numerous inhabitants of the endemic area proves (114, 115). While the overwhelming majority of cases of histoplasmosis heals, others develop single lesions, so-called "tuberculomas", which for many years were diagnosed as granulomas, "etiology unknown", until PUCKETT (92) and others (21, 37, 85, 134) demonstrated that the cause is *Histoplasma capsulatum*. Other cases become chronic and develop cavitary lesions which in all aspects mimic tuberculosis with which they are generally confused (40, 80, 86). In childhood, especially, fulminating cases are seen which develop hepatosplenomegaly, leucopenia and severe anemia and waste away in a relatively short time (1, 75, 118). Several reviews on histoplasmosis have appeared (71, 87, 110, 118), and the clinical and radiologic picture has become the subject of innumerable publications (5, 8, 13, 18, 20, 27, 32, 34, 36, 37, 38, 39, 44, 57, 58, 59, 60, 62, 67, 72, 75, 76, 90, 117, 123, 124, 127, 130, 135). Some reports show little concern about the verification of the diagnosis (16, 17). The nutritional requirements of the organism have been studied (88, 89, 101, 107) and new media and methods suggested (7, 50, 126). An important addition has been the egg medium of KURUNG (66), which permits the change to and the maintenance in the yeast phase with great ease. Tissue cultures were used successfully (69). The resistance to physical agents has been studied (19, 64, 96). Attempts of immunization were reported (54, 94, 98, 103, 104). Recently, considerable controversy has been aroused by the description of a larger organism by VANBREUSEGHEM which has so far been isolated only from African natives and African monkeys. The question whether *Histoplasma Duboisii* constitutes a separate species or just a variety has not been settled conclusively yet (23). Laboratory infections present a real hazard (22, 93, 120). The use of actidione for primary isolation of *Histoplasma* was suggested (49, 70, 116).

The development of skin testing antigens has been very helpful in the epidemiologic research, especially in establishing the zone of endemicity (4, 25, 74, 81), even if experimentally cross reactions occurred with animals inocculated with *Blastomyces dermatitidis*, *Coccidioides immitis* or *Haplosporangium parvum*.

The same cannot be said, unfortunately, about the value of serologic tests (105, 106, 108, 119) which are much less reliable and sometimes give negative results or cross reactions which greatly impair the diagnostic use of serologic reactions. Attempts at treatment have not been successful with a host of remedies, but lately antifungal agents have given new hope based on experimental data in animals (11, 24, 28) and men. Morphologic variations in tissue (6, 78, 125) and culture (79) including giant forms in necrotic material and in tissue explants (111, 129) were described. Experimental inocculation into animals gave pathogenetic information (51, 99, 100).

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SPOROTRICHOSIS

Multiple budding of the yeast form of *Sporotrichum schenckii* and an "abortive yeast phase" were demonstrated by SALVIN in liquid medium (19). The latter was transitional between the yeast and the mold form of the organism.

Intraperitoneal inoculation of mice leads to progressive sporotrichosis(1). Cultures were easily obtainable from the organs at autopsy. Injection of the organisms into the paw of the animal lead to localized disease with a good deal of local reaction (1). In experimentally infected mice soluble antigens have been demonstrated (15). It has also been reported that cross reactions occur between an anti-*Sporotrichum schenckii* serum and pneumococci types 10, 22, 23, 31 and 32. This is also true of the reverse situation, that is between the same types pneumococcal anti-serum and the organism, *S. schenckii* (16).

The finding of the asteroid form of the organism in human tissues has been reported. Though the biopsy seldom yields specific organism structures, the authors state that this should be expected in the occasional case (12, 13, 18).

The existence of true systemic sporotrichosis can be questioned in the three reported cases (3, 6, 7)

Typical cases of sporotrichosis occurring on the extremities, usuallly the hand and arms have been described by many authors. (2, 5, 10, 11, 20, 22) Unusually located lesions were described on the eyelid (8, 14) and on the side of the nose and thigh (17). Treatment with iodides remains the treatment of choice (14, 21, 24).

The finding of *Sporotrichum schenckii* in salt hay, (*Spartina patens*) which is used on Long Island for the cultivation of tulip bulbs, was associated with several cases of sporotrichosis, many of which lived within a small radius in Suffolk County on the south shore of Long Island (4, 9, 14, 23).

YOUNG & ULRICH described a case of a localized skin lesion from which the organism, *S. Gougeroti* was cultured (25).

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ASPERGILLOSIS—CHROMOBLASTOMYCOSIS—MUCORMYCOSIS

Aspergillosis

An unusual entity, the intracavitary fungus ball, has been described by several authors (17, 21, 26). Masses of mycelia and debris are found inside of a cavity wall usually located in the upper lobes and of long duration. Bronchiectasis has been the usual primary cause of the cavity. Mycelia were noted frequently in the sputum and though cultures were most often sterile *A. fumigatus* was found occasionally (26). One of Gerstl's cases was noted to have invasion of both lung tissue and blood vessels.

Corpe & Cope's case of a resected lung cyst revealed *A. fumigatus* on culture of the cyst (13). In a case of disseminated peribronchial abscesses *A. fumigatus* was cultured and hyphal invasion of the wall of a duodenal ulcer was present as well (36).

Generalized aspergillosis is almost always associated with some demonstrable abnormality of body defense (11, 19, 35, 37).

The pathologic reaction to the fungus can be purulent (35) or granulomatous (11). Jackson et al reported two cases of intracranial granulomatous lesions removed surgically from which aspergillosis was diagnosed morphologically. (22).

Chromoblastomycosis

Essentially typical cases of chromoblastomycosis have been reported by several authors (9, 12, 15, 16, 20, 23, 27, 31). Ten of these were reported from Louisiana, the largest concentration of cases in the United States (20, 31). The most consistently successful treatment was excision of the lesion with or without skin graft (10, 12, 15, 16, 20, 23). 3 cases were reportedly successfully treated with calciferol and iodides (9).

Azulay inoculated *H. pedrosoi* into the skin ot a human volunteer. (1). A typical lesion of chromoblastomycosis developed proven by biopsy and culture but it took a year for the full evolution of the lesions.

Kaess found *H. pedrosoi* sensitive to high doses of deamidines in vitro while Bocobo found both *H. pedrosoi* and *P. verrucosa* sensitive to stilbamidine, propamidine and pentamidine (8, 23). *H. pedrosoi* was the most sensitive being inhibited by 0.01 mg of stilbamidine per ml. of media.

Evidence that *Hormodendrum* species may cause brain lesions has been presented first by Binford (7, 24, 30). The organism cultured from the cases of Binford & Thompson and King & Collette was classified as *Cladosporium trichoides* while the report of McGill & Brueck noted an *Hormodendrum* with no further classification.

Carrion summarized the existing clinical and etiological concepts of the disease, chromoblastomycosis in a report to the New York Academy of Science in which he proposed that all the pathogenic species of *Hormodendrum* should be grouped into one genus, *Fonsecaea* (10).

Mucormycosis

Mucormycosis (4) is found particularly in diabetics (2) and in patients receiving adrenal steroids, antibiotics or both (3, 37). In these cases arterial thrombi especially of the internal carotid have been found made up wholly or partly of mucor hyphae (5, 18, 25, 28, 34).

Martin et al (28) have stated that ophthalmoplegia and signs of cerebral involvement in diabetics in acidosis should direct thought towards thrombosis of the internal carotid artery due to invasion by mucor. Bauer et al (5) found sinusitis repeatedly in addition to meningeal and vascular involvement.

Stefanini & Allegra (33) recently described mucormycosis in a treated leukemia patient while Mayfield & Condie (29) found this entity in a patient with thrombocytopenic purpura.

Baker (3) has recently called attention to intestinal mucormycosis.

Experimentally alloxan diabetes was demonstrated as an essential factor in the pathogenesis of mucormycosis (6, 14, 32). This was especially prominent during the acute toxic phase of alloxan diabetes. There was little difference noted in the character of the experimental infection when the organism was inoculated into the chronically diabetic animal (32).

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MISCELLANEOUS

Rare cases with cultural proof for *Geotrichum* were found from blood (6) and from the bone marrow (43). Positive skin tests for geotrichosis were encountered in a few cases of respiratory illnesses in children (50) which were used to diagnose geotrichosis in a larger group (49). Kunstadter had previously described a case of bronchopulmonary geotrichosis in an adult (51). Minton et al. (65) reported two cases of endobronchial lesions, one of which was adequately proven. One case of pulmonary disease with infiltrative chest x-ray findings was described by Good (29).

Cases of meningitis due to *Allescheria boydii* were described in detail (4, 7). *A. boydii* was found in the sputum of a patient six times but no morphological verification of the pathogenic role of the fungus in the lung disease was given (17). This organism has been cultured from the soil (3) and was pathogenic for mice when mixed with gastric mucin (1).

According to Neves & Costa tinea nigra in the United States is due to *Cladosporium wernecki* while in the Orient the cause is *Cladosporium mansoni* (69). These same authors reported successful treatment of a typical case with iodine, salicylic acid and alcohol. *Cladosporium wernecki* was sensitive in vitro to stilbamidine, propamidine, pentamidine, diethylstilbestrol, methyl testosterone and isonicotinic acid hydrazide (11).

Neuhauser's case of maduromycosis had black granules in the pus and in biopsy material (68). The organism cultured was *Madurella grisea*. Apparent healing resulted from use of 4, 4' diaminodiphenyldulfone. Cases of "madura foot" have been reported due to a variety of organisms including *Monosporium, Madurella, Nocardia asteroides*, etc. (14, 31, 37, 48, 62, 64).

A review article on S. A. blastomycosis informs (24) about the (mostly brasilian) literature, pointing out that the involvement of the gastrointestinal (including the oro-pharyngeal) mucosa is prominent; at the same time it has become obvious that pulmonary involvement is much more common than formerly believed and the (pulmonary) portal of entrance may turn out to be the main route of infection and identical with N. A. blastomycosis.

Fungus keratitis was reported after use of corticosteroids in ocular infections (54, 95). Experimentally 80% of rabbits could be infected with fungi in presence of corticosteroids in contrast with 20% infections in the controls (53).

Mycoses in animals

Several reviews on veterinary mycology have appeared (27, 30, 84, 85).

Mycoses are common in animals but only few well documented cases per species are on record. *Microsporum* species especially *M. canis* were found commonly (27) in kittens, puppies and transmitted to (mostly young) humans. *Trichophyton* species were found in guinea pigs, cattle, horses, poultry (27), etc. The essentially anthropophilic *Microsporum audouini* was found in a dog and in a monkey (67, 87). In the Southwest of the U. S. large numbers of dogs (16, 41, 89) and cattle suffer and/or die of coccidioidomycosis, and the organism has been also isolated from a large number of other lower animals (91). Infection with *Coccidioides immitis* and *Histoplasma capsulatum* occurs in animals by inhalation of spore containing "soil" as in men. Skin testing of animals in the endemic areas of coccidiodomycosis and histoplasmosis has given results very much comparable to the ones obtained in human. Histoplasmosis is common in dogs in the endemic area (15, 60, 66, 78, 79, 80) and was found in 50% of trapped dogs and cats in Laudoun County and personal experience tends to agree with these figures (21, 81, 86). Haplomycosis was reported in Montana rodents (42). Cryptococcosis turned out to be a serious epidemiologic problem in cattle, where large outbreaks of mastitis occurred (73). Spontaneous cryptococcosis was also reported in dogs, cats, and monkeys (38, 88, 93). The isolation of *Cryptococcus neoformans* from pigeon droppings and the soil is one more indication of the importance of veterinary mycology (22). Aspergillosis continues to be an important cause of disease especially in birds in captivity and in poultry (19, 39). Actinomycosis is notoriously common in cattle, but the anaerobic *A. bovis* and the aerobic *Nocardias* were observed in several other animal species (5, 12, 61). Blastomycosis was found especially in dogs (57, 75, 77).

Actinomycosis and nocardiosis

The disease caused by the anaerobe *Actinomyces bovis* is seen in all localities but not commonly. Onisi (70) confirmed the fact that the tonsils, peridental spaces and other parts of the oral cavity normally harbor the organism. The organism becomes invasive after trauma, either accidental or surgical, to the oral cavity. How the organism gets into this locale is not understood since it has not been cultured from natural sources (20). Pathogenicity studies may be done in the hamster (36) and in the mouse (63) but in the latter the organism must be combined with mucin. Most of the recent work has been directed towards evaluation of antibiotic treatment (35), and excellent results have been reported with penicillin (25, 83), aureomycin (25, 59) and terramycin (52).

Nocardiosis has been repeatedly reported in the 10 year period, in men (8, 10, 34, 37, 72, 96, 100), and animals (9, 12) but is by no means a common disease in the U. S. The acid fast character of the most commonly found *N. asteroides* and the tendency to fragment into bacillary forms produce a differential diagnostic problem with *M. tuberculosis* (2, 28, 66). McQuown (58) has mentioned without documentation that $5^0/_0$ of all hospitalized patients with "tuberculosis" have nocardiosis. Pathogenicity studies were not always conclusive. The inoculation of the yolk sac of embroyonated chicken eggs is considered by some authors as a good culture medium, which simultaneously establishes the identity of the *Nocardia* (13). In view of the widespread presence of *Nocardias* in nature the interpretation of positive clinical cases should be very critical. Meningo-encephalitis (18, 23, 40, 44, 47, 56, 76, 97) is a common complication of the disease and the same can be said about nephritis. Association with tuberculosis and lymphomas has been noted (23, 100). The treatment is unsatisfactory even if occasional cases are reported improved inconsistently with sulfadiazine, penicillin and broad spectrum antibiotics (82, 92, 94).

The great difficulty of a classification of the Actinomycetales is evident from Waksman's monographs (98, 99).

Technique

A simple but elegant method for obtaining single spores from surface cultures was described by Georg (26), a method for preparation of permanent stained culture mounts by Kligman (45).

Few methods have contributed so much to progress in medical mycology as the development of specific staining techniques in histopathology. The first such stain (BAUER) introduced by LILLIE (55), was followed by the periodic acid-SCHIFF reaction (46), the GRIDLEY (32) procedure and finally by GROCOTT'S (33) modification of GOMORI'S methenamine-silver stain. The PAS stain and especially the two latter stains permit the recognition of small amounts and often dead organisms, which cannot be demonstrated by any other method (71, 90, 101).

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DERMATOPHYTES

Microsporum audouini has become the most common cause of tinea capitis in the United States (3, 10, 13, 17, 19, 29, 43, 58, 67, 73, 79, 85). Much less frequently, *M. canis* proved to be the etiologic agent in some areas in the Southwest (51, 65, 67, 79) and East (83). Infection of the scalp due to *M. gypseum* is rare (12, 74, 84).

Trichophyton tonsurans infections have become an important public health problem in the United States since 1950. Cases were reported from Texas (41, 51, 64), California (44, 66) and other southwestern states (64). Recently cases have been reported from Michigan (36, 80), Ohio (78), Midwest (59) and the East (18, 48). The disease is believed spreading into the southwestern states from the endemi carea in Mexico and into New York from Puerto Rico (24).

Small epidemics of *T. verrucosum* occurred in farming communities in Central Pennsylvania (16) and Iowa (9). Other reports concerned outbreaks in New Hampshire (42), New York (12, 72) and Michigan (27). *T. verrucosum* infections are directly related to contacts with infected cattle.

From time to time endemic foci of *T. schoenleini* infections have appeared in various parts of this country (14, 15, 68). *T. violaceum* was reported once from Illinois (33).

Interesting *Trichophyton rubrum* infections were reported associated with nevoid anomalies of skin (2, 69, 70), systemic lympoblastoma (52) or nodular granulomatous perifolliculitis of the legs (87).

The commonest cause of athlete's foot reported was *Trichophyton mentagrophytes*, and the next most common was *T. rubrum* (7, 8, 35, 40, 54).

Trichophyton mentagrophytes caused ringworm among thousands of guinea pigs (57). Cats and dogs were frequently infected with *M. canis* (56). *T. mentagrophytes* and *T. verrucosum* were the most common causes of ringworm in horses and cattle. Transmission to men was evident (9, 16, 27, 56).

Since the scalps of adults are nonsusceptible to *M. audouini* infection, ROTHMAN et al. (71) studied the local changes in the scalp following puberty and found low boiling saturated fatty acids with selective fungistatic action on *M. audouini*. Highly active normal aliphatic monobasic acids having odd numbers of carbon atoms were isolated from hair fat of adults. This fat prevented infection of new hair which grew after the old infected hair fell out. However KLIGMAN & GINSBERG did not confirm this (49).

KLIGMAN (46, 47) described the pathology of tinea capitis due to *M. audouini* and *M. canis* by inoculation of these organisms in to volunteers and studying biopsy material.

Growth factors essential to the development and formation of macroconidia of *M. audouini* were found to be present in yeast extract and are produced by *Bacillus weidmaniensis* (37, 38, 50). Temperature at 25 to 27° C. also promotes the development of macroconidia in yeast extract honey agar (39).

After studying the relationship between the downy and granular forms of *T. mentagrophytes*, GEORG (27) concluded that they represent two forms of a single species. The downy type culture has less virulence for experimental animals and is most commonly isolated from chronic, low grade infections whereas the granular type culture is most commonly isolated from acute suppurative lesions, particularly those acquired from animals (77).

The new variety, *T. mentagrophytes* var. *nodular*, is a mutant with brilliant orange-red pigment. It was isolated in nine cases of ringworm of the skin and nails from various parts of the United States (30).

The development of macroconidia in *T. rubrum* is enhanced by the addition of tryptose to heart infusion agar. Pigment formation by *T. rubrum* depends not only on the presence of dextrose, but also on the source of nitrogen (5, 34). Corn meal dextrose agar can be used as an aid in differentiating *T. mentragrophytes* from *T. rubrum* (5).

Trichophyton album, *T. discoides* and *T. ochraceum* are considered variants of a single species *T. verrucosum* (4, 20, 53). *T. verrucosum* is found to require inositol and thiamine for growth (16, 20, 53).

The growth of *T. schoenleini* was not enhanced by vitamins (20). *T. violaceum* showed some growth stimulation when thiamine was added to the medium. Further stimulation of growth was observed with the addition of casein or peptone (21). On this medium *T. violaceum* rapidly developed micro and macroconidia (22).

Addition of thiamine to medium enhanced the growth of *T. tonsurans* only slightly (26, 81, 82), *T. megnini* and *T. gallinae* were established as distinct species (23, 76).

Keratin digestion by dermatophytes was demonstrated (61, 62). Isolation of *M. gypseum* from soil has been accomplished (1, 31, 32).

The fungistatic or fungicidal effects of many compounds were tested. Tetrachloroparabensoquinone (30), Triacetin (55), 6-iodo-thymol (11), a number of nitrosteurenes (6), N-butyl-3-phenyl-salicylamide (45, 65), N-phenyl-3-phenyl-salicylamide (63), parabens (75) and many others were found to be effective in vitro.

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LIBRI NOVI

OBRTEL, JAN and Co-workers: *ONEMOCNĚNÍ VYVOLANÁ KVASINKOVITÝMI MIKRO-ORGANISMY* (Diseases caused by fermenting microorganisms). Praha, 1956, Státní zdravotnické nakladatelství. 207 pages. Illustrated. (Czechish).

Those who know the outstanding color atlas of "Dermatophytes" (Praha, 1950) of the senior author could expect something substantial in the presentation of this new monograph on diseases caused by yeast-like organisms. The senior author secured the co-operation of 15 co-workers. Chapters cover the morphology, biochemistry, cultural method of *Candida albicans* (KOCKOVÁ-KRATOCHVILOVÁ); experimental Candida infections (JOHN, SCHINDLER, VORREITH); antibiotica and candidiasis (JOHN and SCHINDLER); systemic infections by *candida* (TOMAN and JOHN); pathologic anatomy of infections by yeast-like organisms (VORREITH); small chapters on localized infections by *Candida* (HRADCOVÁ; TOMAN; TRÄGEROVÁ); vaginal candidiasis (PETER). OBRTEL contributed the chapters on candidiasis of the skin and nail organs. There is a chapter on occupational candidiasis (MIKULECKY; FRÁGNER and SVATEK; HÜBSCHMANN and FRÁGNER). A short chapter finally deals with the deep seated mycoses: North- and South American blastomycoses; coccidioidomycosis; histoplasmosis, chromoblastomycosis.

As far as the discussion of *Candida albicans* as a yeast-like organism is concerned, its morphology, biology, biochemistry are covered satisfactorily. They are up to date. However, when it comes to the clinic of "candidiasis" their authors are completely lost. Particularly regrettable is the presentation of OBRTEL on account of the most serious diagnostic errors. One could say, it is the most complete collection of errors beautifully illustrated with excellent black and white photographs. The clinical conditions depicted as "candidiasis" include typical cases of seborrheic dermatitis in its different localizations, erosio interdigitalis blastomycetica, nummular eczema, Lichen Vidal, infantile eczema, pompholyx of the nail organs, and in order not to miss anything, the "monilial granuloma" of HAUSER and ROTHMAN (a case of multiple congenital epidermal defect). The etiology of all these entities is already sufficiently clarified to be other than "Candidiasis". In all these instances *Candida albicans* is a poor commensal, a nosoparasite, luxuriantly vegetating in the serum drenched epidermal detritus. At the present state of far advanced etiologic research in these clinical entities such grave errors should not be incorporated in an otherwise commendable presentation. The reviewer repeatedly advised that two signs should be displayed, as a stern warning against such errors, in every mycological laboratory. One should read: even a repeated fungus culture **in itself** is no proof of its etiologic significance. The other one should read: "I saw a rabbit climb on a tree, if you don't believe it, I'll show you the tree". The chapters on deep seated mycoses do not belong in a monograph under the title given, since the cultural phase of all of these causative organisms is not a yeast or yeast-like organism, but clear cut hyphomycetes.

The monograph is illustrated with 120 black and white, clinical and mycological photographs and photomicrographs of outstanding quality. Particularly excellent are the photomicrographs with phase microscopy. Every chapter has its own detailed bibliography considering the world literature. For the Western World it is valuable that the Russian literature is also included. The manufacturing of the book: paper, printing, binding is outstanding. Useful index is attached. The reviewer deeply regrets that this monograph as a whole cannot be recommended due to its manifold, grave, clinical-etiological errors.

TIBOR BENEDEK

ANNOUNCEMENT

A seminar on "Histoplasmosis" sponsored by the Jewish Hospital Association, Cincinnati will be held February 11, 1958 in Cincinnati. The invited participants in this seminar are active workers and leaders in the field of mycologic research. A limited number of mimeographed transcripts of the proceedings will be available on request.

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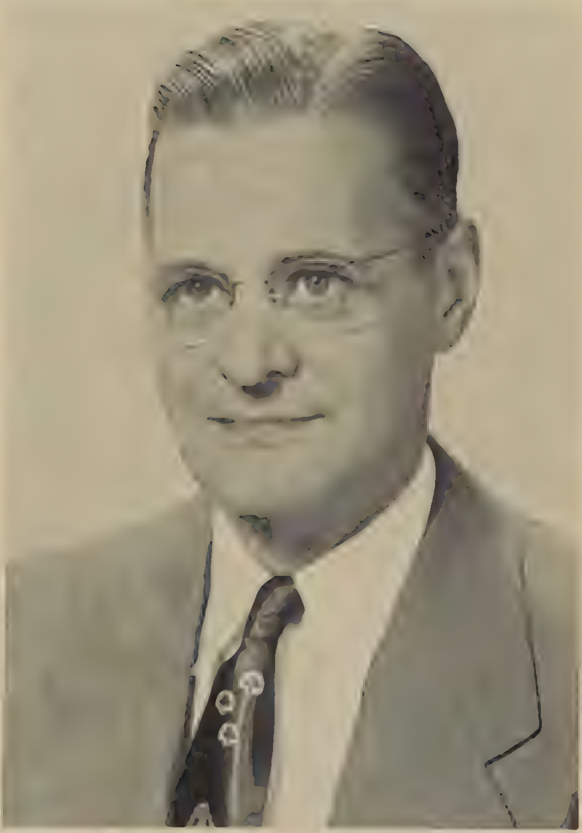

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